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Human Immunoglobulin E

Structural Studies on the Epsilon Chain ND

by

Hedvig von Bahr-Lindström

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HUMAN IMMUNOGLOBULIN E
STRUCTURAL STUDIES ON THE EPSILON CHAIN ND

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Uppsala 1974

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STRUCTURAL STUDIES ON THE EPSILON CHAIN ND

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Hedvig von Bahr-Lindström

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NOMENCLATURE

Immunoglobulins are proteins of animal origin endowed with known antibody activity, and certain proteins related to them by chemical structure and hence antigenic specificity. Related proteins for which antibody activity has not been demonstrated are included - for example, myeloma proteins, Bence-Jones proteins and naturally occurring subunits of the immunoglobulins (WHO, 1964).

Immunoglobulin classes. Five classes are known: IgG, IgA, IgM, IgD and IgE and within each class two types: K and L (WHO, 1964).

Polypeptide chains. The immunoglobulin molecules consist of two kinds of polypeptide chains: the class-specific heavy (H) chains (γ-, α-, δ-, μ- and ε-chains for the respective classes) and the type specific light (L) chains (κ- and λ-chains for the respective types).(WHO, 1964).

Enzymic fragments. The term fragment is reserved for portions of the molecules obtained as a result of the cleavage of peptide bonds. The "classical" fragments are: the peptic fragment $F(ab')_2$ and the two papain fragments Fab (antigen-binding) and Fc (crystallizable) (WHO, 1964). Fab consists of the N-terminal portion of the H-chain, Fd, linked by a disulphide bond to the L-chain. $F(ab')_2$ constitutes a disulphide linked dimer of the fragment Fab' which is slightly larger than Fab. Fc is the disulphide linked dimer of the C-terminal portion of two H-chains (see also Fig. 1a).

Homology (domain) regions. The heavy and light chains of an immunoglobulin molecule consist of compactly folded homology regions which contains about 110 residues stabilized by a single intra-chain disulphide bond and are connected to neighbouring regions by less tightly folded stretches of polypeptide chain (Edelman et al., 1969). The number of domains are two (V_L and C_L) in the L chains and four (V_H, C_H1, C_H2, and C_H3) in the H chains, excepting the μ- and ε-chains which in addition contain a C_H4 domain (Watanabe et al., 1973, Bennich & Johansson,

1970). The domains are regarded as functional units for the different biological properties of the immunoglobulines. The V_H and V_L domains execute the antigen binding functions and the C_H domains execute functions such as complement fixation, skin fixation and placenta passage of the molecules.

Abbreviations

A_{280} = absorbance at 280 nm

CHO = carbohydrate

cpm = counts per minute

DCC = diphenylcarbamyl chloride

Dansyl = 1-dimethyl-aminonaphthalene-5-sulphonyl chloride

DFP = diisopropyl phosphofluoridate

DTT = dithiothreitol

e = charge

E:S = enzyme to substrate ratio

Fluram = fluorescamine

HVE = high voltage electrophoresis

Mw = molecular weight

m = mobility on HVE relative to free aspartic acid

PTH = phenylthiohydantion

TMS = trimethylsilyl

V_e = elution volume

V_t = total volume

C-terminal = carboxyterminal

N-terminal = aminoterminal

INTRODUCTION

Immunoglobulin E constitutes a minor but distinct class of serum immunoglobulins in man and other species, which is responsible for various hypersensitivity reactions of the immediate type (for reviews see: Bennich & Johansson, 1971; Ishizaka & Ishizaka, 1971).

Ishizaka concluded from extensive studies on the antigenic structure of reagins (Ishizaka et al. 1966a, 1966b) that the reagins belonged to a new class of human immunoglobulins, differing from IgG, IgA, IgM and IgD. Confirmation for the existence of IgE was provided by Bennich et al. (1969), who showed that the antigenic and biological properties of reagins could be ascribed to a unique myeloma protein IgE(ND) isolated by Johansson and Bennich (1967) and its normal counterpart (Johansson et al., 1968).

As the serum level of IgE is extremely low (less than 1 μg/ml in normal serum) the discovery of IgE myeloma protein was a prerequisite for investigation of the structural characteristics of this immunoglobulin class. Since IgE(ND) was found in 1965 in Uppsala only 4 IgE myeloma proteins (Ogawa et al., 1969, Fishking et al., 1972, Nezlin et al., 1973, Stoica, G., personal communication) have been identified and partially characterized. The general characteristics of IgE(ND) which is the protein described here can be broadly summarized as follows (for review see Bennich & Johansson, 1971).

IgE(ND) is of type L and the yields of heavy (ε)

and light (λ) chains obtained after reduction are consistent with a four chain structure having a molecular weight of nearly 190 000. By taking into account the carbohydrate content, which is about 12 % for intact IgE, the polypeptide portion of the epsilon chain is significantly larger (approximately 11 000) than the gamma chain and approximately the same size as the mu chain, i.e. 62 000 for ε(ND).

The general structure of IgE(ND) can therefore be described in terms of the basic structure for an immunoglobulin molecule as originally postulated by Porter (1962). A diagrammatic representation of this structure is shown in Fig. 1a, including the distribution of homology regions, or domains, (Edelman & Gall, 1969) and the disposition of the classical proteolytic fragments (Cohen & Porter, 1964).

IgE(ND) carries no known antibody activity but the myeloma protein has retained the skin-fixing (i.e. basophil(mastcell) binding) properties that are characteristic for the IgE class. This cytotropic activity which is a function of the constant portion of the epsilon chain has been located to the Fc region (Stanworth et al., 1968).

Proteolytic cleavage of IgE with papain and pepsin produces Fc and $F(ab')_2$ fragments (Bennich & Johansson, 1967) which are larger in size as compared to the classical fragments (see Fig. 1a) produced from IgG. A related fragment Fc" (Bennich & Johansson, 1970) previously designated Fc-like (Bennich & Johansson, 1967) or Fc′

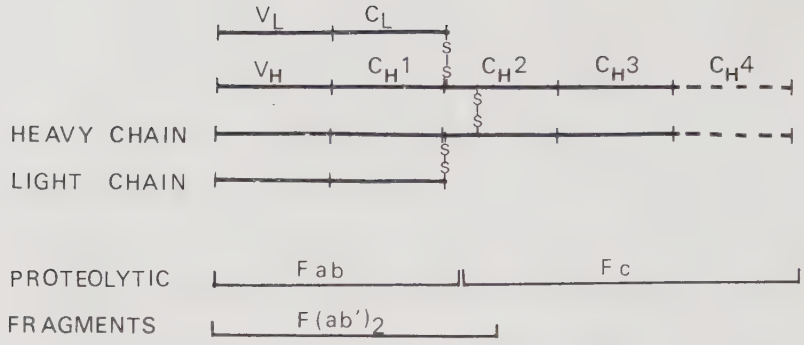


FIG.1a. Schematic structure of immunoglobulins which illustrates the location of proteolytic fragments (Cohen & Porter, 1964) and the distribution of domains (Edelman, 1969). Note that the number of C_H regions varies with the Ig class.

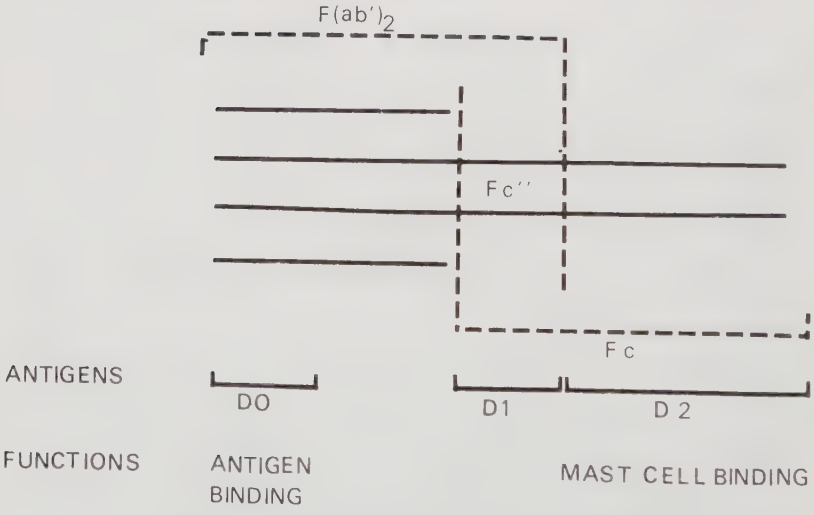


FIG.1b. Schematic structure of IgE(ND) showing the molecular location of the fragments $F(ab')_2$, Fc and Fc" and their biological functions.

(Ishizaka et al.,1970) has also been found. Antigenic analysis and physicochemical and biological studies of $F(ab')_2$, Fc and Fc" (Stanworth et al., 1968) have revealed that $F(ab')_2$ and Fc contain an overlapping segment which corresponds to Fc" as shown in Fig. 1b. Taking into consideration also the molecular size of the heavy chain these results suggested the possibility that the constant part of the epsilon chain comprises 4 homology regions. (Bennich & Johansson, 1970).

The aim of the present study was to determine the primary structure of that part of the heavy chain of IgE(ND) which is common to all IgE molecules, i.e. the constant region and compare this with the sequence in the homologous regions of the γ- and μ-chains.

MATERIALS

Isolation of IgE, $F(ab')_2$ and Fc"

IgE myeloma protein was isolated from the serum of patient ND by salting out (Na_2SO_4) and further purified by ion exchange chromatography and gel filtration as described by Bennich and Johansson (1971).

$F(ab')_2$-ε produced from IgE by digestion with pepsin (1%) at pH 4,5 (0.1 M Na-acetate), was isolated by gel filtration (Fig. 2a) on Sephadex G-150 at neutral pH (Bennich & Johansson, 1971).

Fc"-ε was isolated from either IgE or $F(ab')_2$-ε after digestion with pepsin (1%) at pH 2 (0.01 M HCl) for 10 hours at 37°C. The fragment was purified by ion exchange chromatography and gel filtration on Sephadex G-150 at neutral pH (Fig. 2b) and rechromatographed on

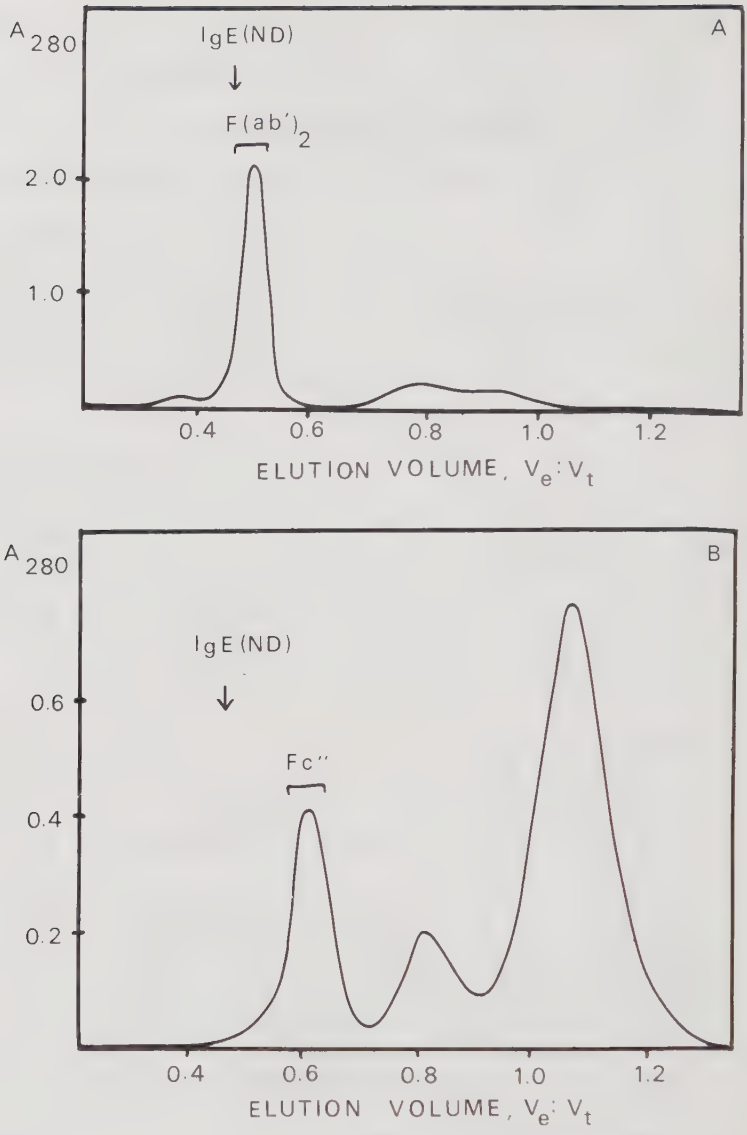


FIG. 2 . Gel filtration on Sephadex G 150 (3.2 x 90.0 cm) in 0.1 M Tris-HCl-0.2 M NaCl-0.002 M EDTA-Na_2 pH 7.7 (flow rate 2 cm/h, fractions 3.6 ml/tube of: A, peptic digest (E:S = 1:100, pH 4.5, 10 h) of IgE(ND) and B, peptic digest (E:S = 1:100, pH 2, 10 h) of $F(ab')_2$-ε.

Sephadex G-50 in 1 M acetic acid.

The purity of IgE, $F(ab')_2$ and Fc" was determined by immunodiffusion, immunoelectrophoresis and starch gel electrophoresis as described (Johansson & Bennich, 1967).

Chemicals

1-^{14}C and ^{3}H labelled iodoacetic acid was obtained from New England Nuclear Corp. Free iodine was extracted with chloroform from a buffered solution of the reagent prior to use. Unlabelled iodoacetic acid (Fluka AG) was recrystallized from petroleum ether. Buffered solutions were extracted with chloroform.

1-Dimethyl-aminonaphthalene-5-sulphonyl chloride (Dansyl chloride) and dansyl amino acid references were obtained from BDH.

Dithiothreitol (DTT) was purchased from Sigma Chemical Company.

Fluorescamine (Fluram^{R}) was obtained from Hoffman-La Roche.

Hexamethyldisilazane and trimethylchlorosilane were obtained from Pierce Chemical Company.

Phenylisothiocyanate and trifluoroacetic acid were of "Sequanal Grade" (Pierce Chemical Company).

Polyamide thin layer sheets were purchased from Chen Chin Trading Company, Taiwan.

Pyridine (Analytical Grade, Mallinckrodt) was re-destilled over KOH and stored at -20°C.

RP 14 Xomat film (35x35 cm, KODAK) was used for

autoradiography. Starch-hydrolyzed for gel electrophoresis was obtained from Connaught Medical Research Lab., Toronto.

Visking dialysis tubing 23/32" (Union Carbide) was soaked in 10 % acetic acid and kept in the cold in the same solvent.

Varsol (Isopar H) was purchased from ESSO Chemicals. All other chemicals were of analytical grade.

Enzymes

Twice crystallized pepsin (Swine stomach) was obtained from Worthington Biochemical Company. Trypsin-Minimal Chymotrypsin (DCC treated) from bovine pancreas was purchased from P-L Biochemicals Inc. Crystalline Chymotrypsin was obtained from Mann Research Laboratories. Thermolysin was obtained from Merck, Darmstadt. Carboxypeptidase B (DFP treated) from hog pancreas was obtained from Worthington Biochemical Company.

METHODS

Reduction and alkylation

Partial reduction of IgE (30 mg/ml) and Fc" (10 mg/ml) in 0.3 M Tris-HCl pH 7.6, 2 mM Na_2EDTA was performed at 20°C for 60 minutes under nitrogen using 10 mM (final conc.) dithiothreitol (DTT).

Total reduction of IgE and Fc" (10 mg/ml in 0.3 M Tris-HCl pH 7.6, 6 M guanidine-HCl) was performed at 20°C for 120 minutes with 10 mM (final conc.) DTT.

The reduced samples were alkylated with iodoacetic acid at 0°C (icebath) for 60 minutes under nitrogen

in the dark. A 10 % molar excess of iodoacetic acid over DTT was used. The specific activity of the ^{14}C- and ^{3}H-iodoacetic acid preparations was 0.2-1 mCi/millimole and 10 mCi/millimole respectively.

After gel filtration the samples were gel filtrated on a Sephadex Column in acid buffer (see below).

Identification of inter- and intra-chain disulphide bonds

In order to distinguish intra-chain disulphides from inter-chain disulphides two methods were used: 1) After total reduction of a partially reduced-^{14}C-alkylated sample, one portion (A) was treated with unlabelled iodoacetic acid and a second portion (B) was alkylated with ^{14}C-iodoacetic acid. The subsequent treatment of portion A and B was identical.2) After total reduction of a partially reduced-^{14}C-alkylated sample alkylation was done with ^{3}H-iodoacetic acid.

Isolation of heavy and light chains

Partially reduced IgE - alkylated with ^{14}C-iodoacetic acid - was fractionated on Sephadex G-100 (3.2x90.0 cm) in 1 M acetic acid at room temperature. The flow rate was 2 cm/hour. Fractions of 4 ml were collected for analysis. Fractions containing heavy (ε) and light (λ) chains were lyophilized.(Fig. 3a).

Cyanogen bromide cleavage

Cleavage of methionyl peptide bonds was performed using CNBr (Gross & Witkop, 1961) in 70 % formic acid (Givol & Porter, 1965) at 20°C. For partial cleavage a CNBr:protein ratio of 1:2 (w/w) was used and the

digestion time was 12 hours. For extensive cleavage the CNBr:protein ratio was 2:1 (w/w) and the digestion time 24 hours. After digestion the samples were diluted 5 times with distilled water and lyophilized.

Enzymic cleavage

All endopeptidase digests were performed at an enzyme to substrate ratio (E:S) 1:100 for 2 to 6 hours at 37°C and terminated by lyophilization unless otherwise stated. Trypsin and chymotrypsin digests were performed in 1 % ammonium carbonate, pepsin in 10 mM HCl and thermolysin in 0.1 M ammonium acetate pH 8.0 containing 5 mM $CaCl_2$.

When carboxypeptidase B was used the reaction was performed in 0.2 M N-ethyl morpholine acetate buffer pH 8.5. One to 20 nanomoles of peptide were treated with 2 % enzyme (w/w) at room temperature and aliquots were taken at 0, 15, 30, 60, 120 minutes and 4, 8, and 20 hours. As controls buffer and enzyme were run in parallel. The reaction was terminated by lowering the pH to about 2.5 by the addition of glacial acetic acid. The aliquots were subsequently evaporated, dansylated and analyzed as described below.

Large peptides or those not migrating during paper chromatography were redigested in situ: The paper strip containing the peptide was sprayed evenly with an enzyme solution (2 mg/100 ml buffer as above) and incubated in a moist chamber at room temperature for 16 hours. The paper strip was dried, stitched on to a new Whatman 1 paper and subjected to HVE and paper chromatography as described under "Purification of peptides".

Performic acid oxidation

Performic acid oxidation of samples containing 10 mg protein per ml was done at 0°C (icebath) as described by Hirs (1956).

Alkylation of methionyl residues

For selective carboxymethylation of methionyl residues, 180 nanomoles of IgE dissolved in 4.5 ml of 5 % formic acid were allowed to react with 170 micromoles (about 60 fold molar excess over methionine) iodoacetic acid (spec. act. 0.3 mCi/millimole) for 50 hours at 37°C (Vithayathil & Richards, 1960). After dialysis against 1 % NH_4CO_3 in the cold the alkylated product was digested with trypsin as described above (Wilkinson, 1969).

Purification of cyanogen bromide fragments

Figure 3 outlines the isolation of the fragments produced by extensive CNBr cleavage of partially reduced-alkylated epsilon chain. The lyophilized digest, dissolved in 15 ml of 1 M acetic acid, was fractionated on Sephadex G-100 (3.2 x 90 cm) in the same solvent. The flow rate was 2 cm per hour at room temperature. Fractions of 4 ml were collected for analysis, pooled as shown in Fig. 3b and lyophilized.

The major peak, A, eluted in the void volume was subjected to total reduction-alkylation and rechromatographed on Sephadex G-100 in 0.2 M Na-formiate pH 3, 6 M urea (Fig. 3c). The fractions were pooled as shown in Fig. 3c, dialysed against 1 mM acetic acid and lyophilized.

Rechromatography of fractions B-H (Fig. 3b,c) was done on Sephadex G-50, 40-80 μ (3.2 x 90 cm) in 1 M acetic acid, 0.15 M formic acid. Pooled fractions were lyophilized.

FIG. 3. Flow sheet for purification of ε- and λ-chains and CNBr fragments.

IgE(ND)

↓ Partial reduction
^{14}C alkylation
G 100 (3.2 x 90.0 cm)
1 M HAc

ε-chain, λ-chain

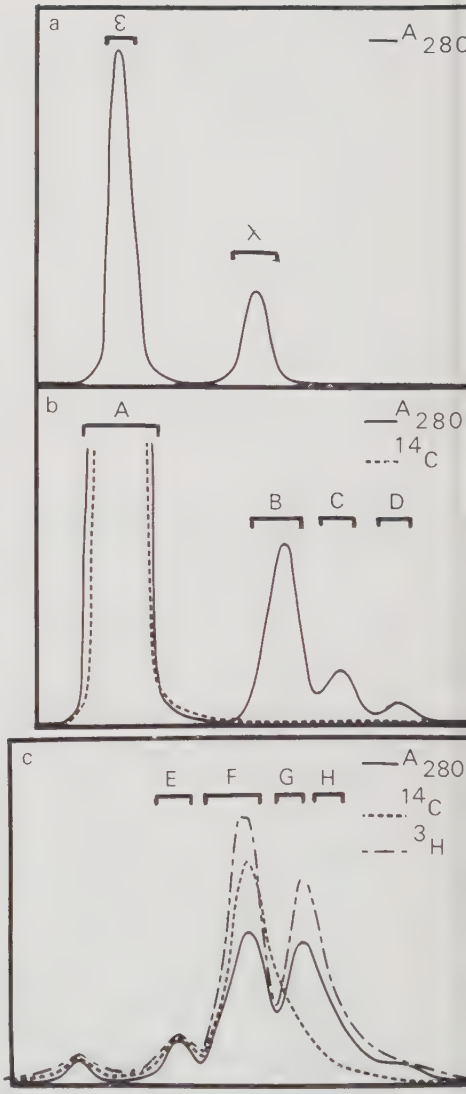
↓ CNBr cleavage
G 100 (3.2 x 90.0 cm)
1 M HAc

A, B, C and D

↓ Total reduction
^{3}H alkylation
G 100 (3.2 x 90.0 cm)
0.2 M Na-formiat-6 M urea

E, F, G and H

Rechromatography
G-50 (3.2 x 90.0 cm)
1 M HAc-0.15 M HCOOH



Fractionation of reduced Fc"ε fragment

Partially reduced-alkylated Fc"ε was separated (Fig. 5) under the same conditions as used for the isolation of CNBr-fragments (Fig. 3b).

Purification of peptides

Isolation of peptides from enzymic digests was performed by high voltage electrophoresis (HVE) on paper and paper chromatography. A maximal load of 0.5 mg digest per cm paper (Whatman 1) was applied. In the first step HVE was run at pH 6.5 (pyridine:acetic acid: water-100:3:897) and 3 kV for 50 minutes. In the second step descending chromatography was run in butanol-acetic acid-water-pyridine (15:3:12:10). In the third step HVE was run at pH 3.5 (pyridine:acetic acid:water-100:10:189) and 3 kV for 60 minutes. When considered necessary any of these steps were repeated. The HVE was performed in a Gilson Electrophorator model D using varsol (Isopar H, ESSO Chemicals) as cooling medium.

For identification of the peptide bands the paper was stained by spraying with cadmium-ninhydrin (3 mM cadmium acetate:1 % ninhydrin in acetone - 15:100). Staining was done on a guide strip (5-10 mm) and the peptide bands were assumed to have migrated parallel to the line of application. Strips containing peptide were cut accordingly. However, low yields and/or contamination of peptides could result if the bands are not parallel to the line of application. Most of these difficulties were overcome by the introduction of Fluram for identification of amino acids and peptides (Undenfriend, 1972). After spraying the chromatogram with Fluram in

acetone (4 mg/l) and drying at room temperature, peptides became visible as fluorescent bands under ultraviolet (365 nm) illumination.

Identification of peptides containing tryptophan and tyrosine was made by inspection of the chromatogram under ultraviolet light immediately prior to staining.

For the purification of radioactive peptides chromatograms were subjected to autoradiography (RP 14 Xomat film, KODAK) for 24-48 hours.

A peptide was considered sufficiently pure when it migrated as a single band in HVE and chromatography. Purified peptides were recovered by elution with 0.1 % ammonia.

Amino acid analysis

Protein or peptide was hydrolyzed in 6 M HCl (containing norleucine as internal standard) in vacuo at 110°C for 18 hours (peptides) and 24 hours (proteins). One to ten nanomoles were analyzed on a modified JEOL JLC-6AH and 0.1 to 3 nanomoles were analyzed on a Beckman 121 M amino acid analyzer. The peaks were integrated either manually or automatically (Autolab Computing Integrator, System AA). The values for threonine and serine obtained for protein hydrolyzates were corrected for destruction during 24 hours hydrolysis using the correction factors 0.95 and 0.89 respectively. Occasionally serine and threonine were poorly resolved on the JEOL-analyzer column which resulted in a too high value for one amino acid and a too low for the

other. Values for carboxymethylcysteine were taken as minimum values due to partial losses that occured either during hydrolysis or at storage prior to analysis. Homoserine lactone in CNBr fragments converts slowly to homoserine in alkaline solution (Ambler, 1965). As no precautions were taken to ensure which form predominated the homoserine value obtained was too low.

Determination of carbohydrate

Monosaccharides released from glycopeptides by methanolysis were converted to trimethylsilyl (TMS) derivatives of methylpyranosides and analyzed by gas liquid chromatography (Clamp et al., 1972).

To 10 to 20 nanomoles of peptide was added a known amount of mannitol (internal standard). The mixture was thoroughly dried in a dessicator (24-48 h over P_2O_5) prior to methanolysis (in 0.5 ml 1.25 M HCl-dry methanol) at 80°C for 24 hours. After neutralization (20 mg silver carbonate) 50 microliters of acetic anhydride was added and the mixture was incubated at room temperature for 15 hours in the dark. After centrifugation the supernatant was transferred with 3x0.5 ml dry methanol to a screw cap (Teflon lined) reaction tube, evaporated and dried over P_2O_5 in a dessicator for 24 hours. Fifty microliters of trimethylsilylating reagent (trimethyl-chlorosilane: hexamethyldisilazane:pyridine - 1:1:5) was added and the mixture was sonicated for 2-3 minutes. One to three microliters were injected into a Pye Unicam model 104

Gas chromatograph equipped with 4-6 feet long glass columns (ID 0,3 mm) packed with Chromosorb WHP-SE 30 (3.8 %).

The carbohydrate content was expressed as moles of monosaccharide per mole of peptide taken for analysis as determined by a simultaneous amino acid analysis.

Endgroup analysis

The amino terminal amino acid residue of proteins and peptides was determined after reaction with dansyl chloride (Gray, 1967) followed by acid hydrolysis and separation of liberated dansyl amino acids by chromatography on polyamide plates (7.5 x 7.5 cm) using the solvents described by Hartley (1970). Between 0.5 and 3 nanomoles of protein (peptide) were taken for each analysis.

For determination of the carboxyterminal amino acid residue, reaction with dansyl chloride was done on a carboxypeptidase digest of the peptide (see Enzymes above).

Amino acid sequence determination

The primary structure of the peptides was determined by the dansyl-Edman procedure as described by Hartley (1970). Between 5 and 50 nanomoles of peptide were taken for each analysis. Dansyl amino acids were separated and identified as described above (Endgroup analysis).

Determination of acids and amides

In order to determine if a dansyl-aspartic or dansyl-glutamic acid could be derived from the respective amide

the method described by Offord (1966) was used. The mobility (m) of the peptide in HVE at pH 6.5 was determined relative to free aspartic acid taking the neutral amino acid band to represent zero mobility. The approximate molecular weight of the peptide (Mw) was calculated using an average residue weight of 110. Using these two parameters the number of charges per peptide could be estimated from the diagram published by Offord. The method cannot be applied to glycopeptides as sialic acid will contribute to the total charge. Furthermore, when the peptide contains histidine the estimation becomes less reliable because of the fractional charge provided by histidine at pH 6.5. For peptides containing cysteine alkylated with iodoacetic acid a corresponding number of negative charges has to be taken into account.

Starch gel electrophoresis

Horizontal starch gel analyses (Wake and Baldwin, 1961) were performed at pH 3 in 50 mM formate, 19 mM NaOH containing 8 M urea (Edelman and Poulik, 1961). Approximately 20 microliter samples containing 1-4 mg/ml were applied to a strip of Whatman 3MM paper (2 x 14 mm) inserted in the gel. A voltage of 14-16 V/cm was applied for 2-3 hours at 8°C. Gels were stained with 0.01 % (w/w) Nigrosin in methanol-acetic acid-water (5:1:4, by vol.) and destained in the same solvent.

Ultraviolet absorbance determination

Determination of ultraviolet absorbance at 280 nm

were carried out on a Hitachi 124 spectrophotometer.

Determination of radioactivity

The ^{3}H and ^{14}C activity was measured in a scintillation counter (Nuclear Chicago model ISOCAP/300). The samples (50-100 microliters) were mixed with 10 ml of scintillation liquid (toluene:Triton X-100 in proportions 1:1). When sequencing radioactive peptides 1.5 ml of the butylacetate used for extraction of the TH amino acid derivative was mixed with 10 ml of scintillation liquid and analyzed.

RESULTS

Cyanogen bromide cleavage of the epsilon chain ND was expected to yield 8 fragments since there are 7 methionine residues per Mw 62 000 of polypeptide (Bennich & Johansson, 1971). Under mild conditions CNBr treatment of partially reduced epsilon chain yields only two fragments of which one (B, Fig. 3b) represents the C-terminal domain with a molecular weight around 11 000 (Bennich et al., 1973).

By extensive cleavage two additional small fragments (C, D, Fig. 3b) are released which indicates that the remaining fragments must be joined by disulphide bridges. This was confirmed by the results of a total reduction of the major CNBr fragment (A, Fig. 3b) which yielded 4 new fragments (E-H, Fig. 3c) resolved by gel filtration on Sephadex G-50.

Similar studies on $F(ab')_2$ and its constituent polypeptide Fd'and on Fc" gave an indication about the relative disposition of the CNBr fragments in the

Fab′ and Fc regions of the epsilon chain: Thus, the Fab′ region contains fragments C(V), D(VI), E(Fd′c), F(Fd′a), H(Fd′t) and the N-terminal portion of G(III); The Fc fragment consists of B(ε4-ε2), G(III), and a large portion of E(Fd′c); The Fc" fragment which represents an overlapping region between Fab′ and Fc contains the C-terminal portion of E(Fd′c) and the N-terminal portion of G(III); and the results of the sequence analyses will be presented accordingly. Designations within brackets refer to the nomenclature used in tables and figures. Starch gel analysis of the CNBr fragments except C(V) and D(VI) are shown in Fig. 4.

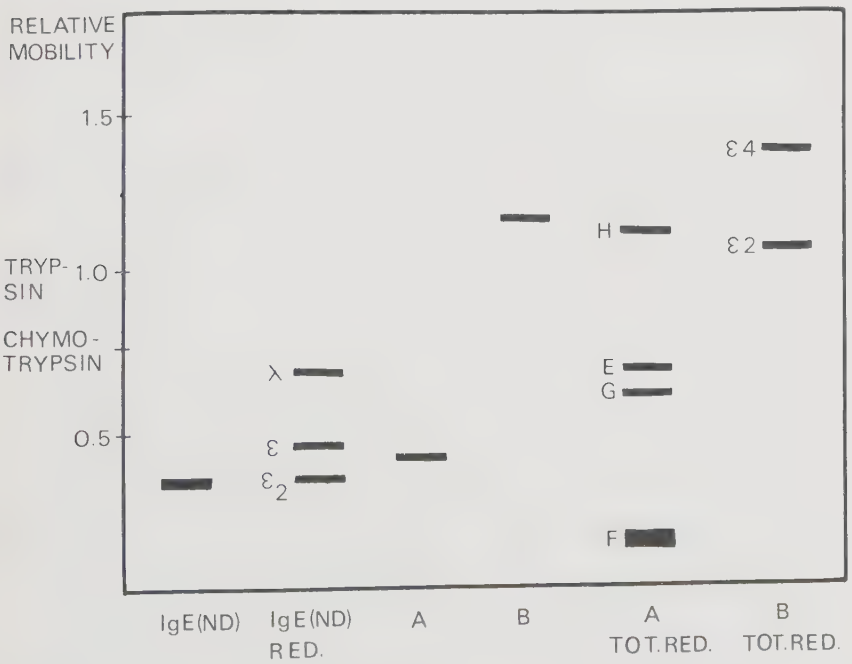


FIG. 4. Starch gel electrophoresis in urea-formate buffer pH 3 of the CNBr-fragments from the ε(ND)-chain compared with IgE(ND), ε(ND)- and λ(ND)-chains.

The primary structure of CNBr fragments in the Fab´ region

Fragment C(V). This fragment which contained carbohydrate and had a strong ultraviolet absorbance at 280 nm consisted of at least 38 residues as determined by amino acid analysis (Table 1). The fragment contained one lysine residue but no cysteine or arginine.

Tryptic cleavage of 600 nmol for 4 hours resulted in two peptides of which one was a dipeptide containing glutamic acid and homoserine, indicating that the C-terminal sequence of the fragment was Gln-Met.

After chymotryptic cleavage of 600 nmol of C(V) for 4 hours 6 peptides were recovered (Table 1, Fig. 6). As none of these peptides contained carbohydrate at least one glycopeptide had been lost.

Peptic digestion of 1 µmol C(V) for 18 hours yielded several overlapping peptides. Only those relevant for the alignment of the chymotryptic peptides are reported in Table 1 and Fig. 6.

The presence of two tryptophan residues in C(V) was indicated by the finding of two groups of peptides having unrelated sequences which both gave a strong ultraviolet absorbance. As tryptophan is destroyed during acid hydrolysis the presence of a tryptophan residue in the sequence will result in the apparent negative reaction whenever tryptophan occupies the N-terminal position. To verify that a "silent" step obtained in the analysis of peptides (P)N1A and (P)B2 indeed represented a tryptophan the small ultraviolet-

-positive peptides (C)N5 and (C)N4 were analyzed either directly with no hydrolysis of the expected tryptophan residue or treated with carboxypeptidase B to release tryptophan in the C-terminal position.

Peptide (P)N1A contained carbohydrate and the composition of the monosaccharides is shown in Table 2. The oligosaccharide chain, CHO:B, is assumed to be linked to an asparaginyl residue in the sequence Leu-Asn-Gly-Thr. Two aspartic and one glutamic acid residue were found in the sequence in agreement with the amino acid composition data (Table 3). The single glutamic acid residue had to be a glutamine as it is present in a basic peptide (C)B4A where the only charge was contributed by the lysine residue (Table 1). As regards the aspartic acids the eventual presence of an amide group in Asp position 4 of the fragment could not be determined due to the charge effect of the sialic acid residue

Fragment D(VI). This fragment migrates as a neutral peptide on HVE at pH 6.5. The fragment contained no carbohydrate, had no ultraviolet absorbance and amino acid analysis indicated that it consisted of at least 11 residues (Table 4). The sequence of the peptide could be determined directly to be:

Thr-Arg-Asp-Ala-Ser-Phe-Ser-Thr-Ala-Tyr-Met

The neutral mobility of the peptide confirmed the presence of an aspartic acid residue.

Fragment E(Fd´c). This fragment which contained carbohydrate and had strong ultraviolet absorbance at 280 nm

consisted of at least 70 amino acid residues, including at least 3 cysteines (Table 5). Direct sequence analysis on the complete fragment showed the N-terminal sequence Ile-Thr-Lys-Arg-Val-.

Tryptic cleavage (300 nmol) for 2.5 hours yielded the expected number of peptides. Two of the peptides (T)NOA and (T)A2A1 contained carbohydrate (Table 5 and 6). The presence of homoserine in (T)A2A1 furthermore indicated that this peptide which contained 40 residues represented the carboxyterminal portion of E(Fd´c).

After chymotryptic digestion (200 nmol) for 3.5 hours 5 peptides were recovered. Due to the limited amount available for digestion the chymotryptic peptides could not be completely sequenced. The amino acid composition of the peptides is shown in Table 5. Peptide (C)N1 contained carbohydrate and one residue of arginine and lysine. Unfortunately this important overlapping peptide was found to represent a mixture of two peptides (C)N1:1 and (C)N1:2 present in the proportions 1:1.6 as deduced from amino acid analysis. Accordingly two dansyl-amino acids were detected in each step of the sequence analysis until step 6 after which (C)N1:2 became silent and (C)N1:1 yielded a phenylalanine.

Despite these difficulties the alignment of the tryptic peptides could be ascertained. For determination of the complete amino acid sequence of E(Fd´c) shown in Fig. 7 peptides obtained in good yields from the Fc" fragment were used as the latter contains a large portion of E(Fd´c).

Peptides (T)NOA, (C)N1, (T)A2A1 and (C)A2A were found to be glycopeptides and the composition of the constituent monosaccharides is shown in Table 6. The oligosaccharide chains, CHO:C and CHO:D, are assumed to be linked to an asparaginyl residue in the sequences -Asx-Asn-Lys-Thr- and -Ile-Asn-Ile-Thr- respectively.

Six aspartic acids and 4 glutamic acid residues were found in the sequence of E(Fd´c). The single aspartic acid residue present in peptide (T)N4A must be the acid as it is present in a neutral (m = 0) peptide containing one lysine residue. The aspartic acid and the two glutamic acids in peptide (C)A3D could not be unambiguously determined with this method (Table 7). The eventual presence of amide groups on the aspartic and glutamic acids in the peptides (T)NOA and (T)A2A1 could not be determined due to the charge effect of the sialic acid residues.

Fragment F(Fd´a). This fragment with the N-terminal sequence Asp-Leu-Arg- consisted of at least 67 amino acid residues including at least 2 cysteines. The amino acid composition is shown in Table 8. Glucosamine indicates that carbohydrate is present.

After tryptic digestion (on 1 µmol) for 18 hours only five peptides were recovered. The sixth peptide was found to be insoluble in the solvents used for peptide purification. The composition of the purified peptides is shown in Table 8.

A chymotryptic digestion for 3.5 hours was performed on 700 nanomoles of F(Fd´a). Only a few of the expected

peptides could be recovered. The composition of the peptides is shown in Table 8.

The tryptic peptide (T)A4B contained one oligosaccharide unit (designated CHO:A, Table 9) presumably attached to an asparaginyl residue in the sequence -Ser-Asn-Ala-Thr-. The same sequence was found in Fc"3, which is one of the constituent fragments of Fc" (see below).

The location of acids and amides is shown in Table 10. As regards the aspartic acids in peptide (T)A4B the eventual amide group at position 1 in the peptide could not be determined because of the charge effect exerted by the carbohydrate side chain.

In Fig. 8 the partial amino acid sequence of fragment F(Fd´a) is shown. The tentative amino acid composition of the segment not yet sequenced is shown within brackets.

Fragment H(Fd´b). The fragment which consisted of at least 65 residues including one cysteine had a strong ultraviolet absorbance at 280 nm and contained no carbohydrate (Table 11). The amino terminal amino acid of this fragment was found to be blocked which indicated that H(Fd´b) could represent the amino terminal portion of the epsilon chain.

Tryptic digestion was performed on 1 μmol for 2.5 hours. Eight peptides were expected to be found but only five could be recovered. One of the isolated peptides contained a Lys-Pro bond which is not split by trypsin, the peptide with the blocked N-terminal amino acid was lost and one peptide containing C-terminal homoserine

was recovered together with peptide (T)N4. The amino acid composition of the purified peptides is shown in Table 11.

As the peptides (T)N4 and (T)N4A represented a mixture of peptides their composition is not given. Extensive purification of (T)N4 gave (T)N5A1 the composition of which is shown in Table 11. Due to the low yield of (T)N5A1, however, the sequence analysis was done mainly on peptide (T)N4. Because of difficulties in removing the impurities in peptide (T)N4A only the first 4 amino acid residues of the peptide could be unambiguously assigned.

The tryptic peptides (T)N3 and (T)B3 were found to have identical sequences which indicated that the difference in mobility must be due to the presence of glutamine in (T)B3 and glutamic acid in (T)N3.

After chymotryptic cleavage of 1 μmol for 3 hours 6 peptides were recovered (Table 11) which accounted for the entire amino acid composition of H(Fd´b) except for 4 residues at the most in the N-terminus.

The peptides (C)B2A and (C)B3A represented overlapping sequences which explains the low yield. Both peptides were released by chymotryptic cleavage of a Leu-Val bond, but whereas (C)B3A had a C-terminal arginine, (C)B2A contained an additional sequence of 8 residues including cysteine and tyrosine as the carboxyterminal as shown by carboxypeptidase B digestion.

The position of a tryptophan residue in the peptides (C)N4B and (C)B1B was confirmed by finding dansyl-

tryptophan when hydrolysis was omitted.

Digestion of 200 nanomoles of H(Fd′b) with thermolysin for 3 hours yielded a great number of peptides of which two provided complementary information (Table 11). Thus, peptide (TL)A3B which had a N-terminal leucyl residue provided an overlap for the chymotryptic peptide (C)B3A, while the mobility of the tripeptide (TL)A3B indicates the absence of an amide group on the glutamic acid residue. The results of the determination of acids and amides in fragment H(Fd′b) is shown in Table 12. The relative position of one glutamic acid and one glutamine residue in (TL)A3B could not be determined, but were placed by homology (V_HI subgroups).

The tentative amino acid sequence is shown in Fig. 9.

The primary structure of CNBr fragments in the Fc region

Fragment B(ε4-ε2). This fragment is produced by CNBr cleavage using mild conditions and consists of two disulphide linked peptides, ε4 and ε2, whose sequences have been reported previously (Bennich et al., 1973). Fragment B represents the C-terminal homology (domain) region of the epsilon chain and the amino acid sequence is shown in Fig. 10.

Fragment G(III). This fragment was found to represent a large carbohydrate containing peptide of at least 152 residues including 4 cysteines (Table 13a). The presence of tryptophan was indicated by a strong ultraviolet absorbance at 280 nm. The N-terminal sequence of the intact fragment was determined to be Asx-Val-

-Asx-Leu-Ser-Thr-Ala-.

From the amino acid composition (Table 13a) at least 19 peptides would be expected to be produced by tryptic cleavage. After tryptic digestion of 500 nanomoles for 4 hours 13 peptides were recovered. One peptide (T)B2A contained a Lys-Pro bond, two peptides (T)N1B and (T)N5 had a lysyl residue both in the N- and the C-terminal position. In addition one peptide, (T)N7, was found to have the C-terminal sequence -Lys-Lys .

Only one peptide (T)N1A contained carbohydrate. After extensive tryptic digestion (500 nmol) using 2 % (w/w) enzyme for 16 hours a second carbohydrate peptide (T)IN2B could be recovered (Table 13a).

Two peptides (T)N8 and (T)IN2B did not contain arginine or lysine or homoserine(lactone) which indicated the presence of an active "contaminant" in the enzyme preparation.

Due to the size of fragment G(III) isolation of overlapping peptides was attempted after digestion of about 500 nanomoles with chymotrypsin (Table 13b), pepsin (Table 13c) and thermolysin (Table 13c) respectively. In addition some of the enzymic peptides, e.g. (T)A2 and (C)A10, had to be further cleaved using a different enzyme to produce smaller peptides (Table 13b,c) suitable for analysis (see under "Enzymatic cleavage").

Fragment G(III) contained 3 tryptophan residues. One tryptophan residue present in the overlapping pep-

-tides (T)N6 and (C)B3B was localized as the C-terminus in the latter peptide by identification of the dansyl derivative without prior hydrolysis. A second tryptophan residue was present in the overlapping peptides (T)A1 and (C)A3C. Its location could not be positively assigned but was assumed to be indicated by the single "silent" step obtained during the analysis of either of the peptides. The localization of the third tryptophan residue, present in peptide (T)N1A, was also done indirectly as described above.

The results of the determination of acids and amides is shown in Table 14.

When a peptide, e.g. (P)A2A, (P)A3A, and (T)A3, contained both acids and amides their relative position could not be determined. They were therefore placed in the sequence by homology as indicated in Fig. 11.

The peptides (T)N1A, (C)N1, (T)N2 and (P)B1C contained carbohydrate and the composition of the constituent monosaccharides in the two oligosaccharide chains, CHO:E and CHO:F, is shown in Table 15. The site of attachment is assumed to be an asparaginyl residue in the sequence -Val-Asn-Leu-Thr- and -Arg-Asn-Gly-Thr- respectively.

The complete amino acid sequence of fragment G(III) is shown in Fig. 11.

The primary structure of the Fc" fragment

Fragment Fc" which originally was defined as an

overlapping segment between the Fab′ and Fc regions by antigenic analysis (Bennich & Johansson, 1971) is characterized by its relative resistance to proteolytic cleavage. Thus, both prolonged digestion of IgE with papain at neutral pH and pepsin digestion at pH 2 will yield Fc" which has retained its antigenic properties. For the present study Fc" was purified from IgE as well as from $F(ab')_2$-ε using prolonged digestion with pepsin at pH 2 (Fig. 2b) as described in MATERIALS.

Fc" which was assumed to represent a covalently linked dimer with an apparent molecular weight of 30 000 as estimated from gel filtration data was found to have a minimum molecular weight of 15 500 as calculated from the amino acid composition (Table 16), excluding the carbohydrate moiety. The presence of 7 cysteines per 15 500 Mw and the findings of Val and Pro as amino-terminal residues indicated the possibility that the fragment was composed of several disulphide linked peptides.

Gel filtration of partially reduced Fc"-alkylated with ^{14}C-iodoacetic acid yielded three radioactive and carbohydrate containing peaks (Fc"1, Fc"2 and Fc"3) as shown in Fig. 5. End group analysis revealed an N-terminal proline residue in Fc"3, whereas the N-terminal in neither Fc"1 nor Fc"2 could be unambiguously determined which indicates that they had to be further purified. Fc"3 which consisted of 15 residues as calculated from the amino acid analysis (Table 16) could be sequenced directly to give: Pro-Leu-Thr-Arg-Cys-Cys-Lys-Asx-Ile-Pro-Ser-Asx-Ala-Thr-Ser. The com-

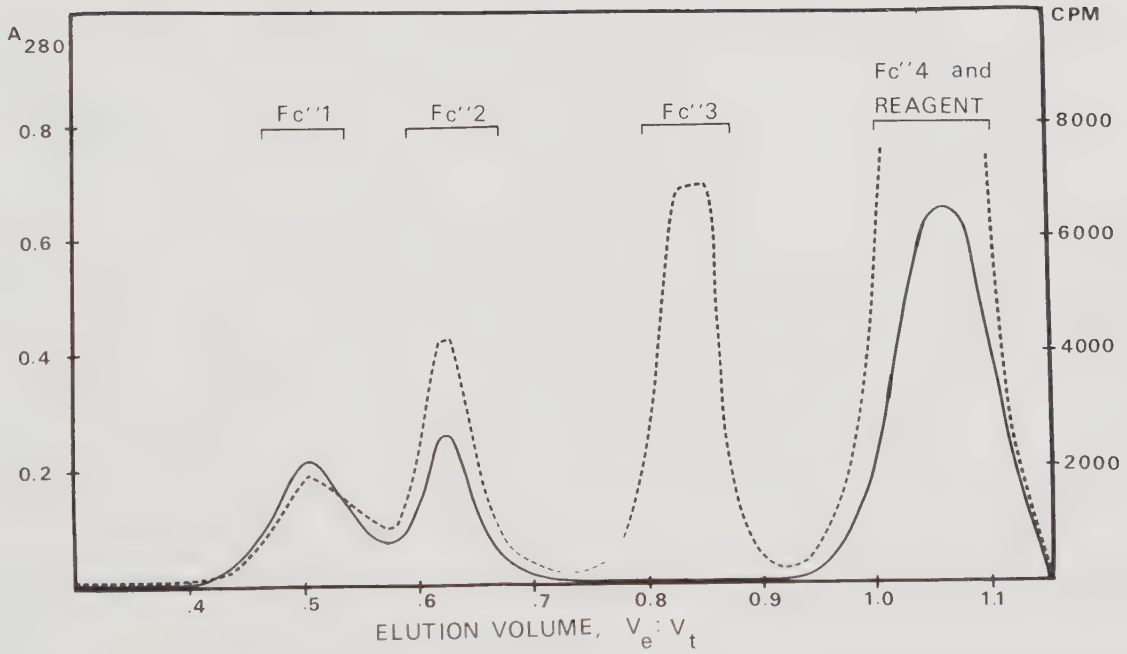


FIG.5 . Gel filtration on Sephadex G 100 (3.2 x 90 cm) in 1 M HAc (flow rate ? cm/h, fractions 3.6 ml/tube) of partially reduced-^{14}C-alkylated Fc". Fractions taken for further analysis are indicated by a horizontal bar. Solid line: A_{280}, broken line: cpm.

position of monosaccharides of oligosaccharide chain (CHO:A) presumably linked to an asparaginyl residue in the sequence -Ser-Asn-Ala-Thr- is shown in Table 9. Analysis of Fc"3 provided an overlapping sequence in CNBr fragment F(Fd'a) between the peptides (T)A3A2 and (T)A4B as shown in Fig. 8.

After performic acid oxidation (on 200 nmol) fragment Fc" yielded 3 distinct components by HVE at pH 6.5.

One peptide with zero mobility was found to be identical with Fc"3 and one of the acid components (mobility 0.4) was found to be similar in composition to both Fc"1 and Fc"2 which indicated the possibility that the latter peptides in fact might be identical. The third component (mobility 1.0) which represented a relatively small peptide containing cysteic acid (Table 16) was not identified after gel filtration on reduced Fc", most likely due to that the peptide was eluted with the reagent peak. Fc"4 was found to represent a pentapeptide with the sequence Pro-Thr-Glx-Cys-Ser , which is identical to the C-terminal sequence of the λ-chain (Putnam, 1967).

As shown in Table 16 the amino acid composition of Fc"1 and Fc"2 was found to be very similar. As the sum of the residues in Fc"2, Fc"3 and Fc"4 was found to be in close agreement with the number of residues in fragment Fc", Fc"1 was likely to represent a disulphide linked dimer of Fc"2.

Fc"2 which consisted of at least 121 residues including a minimum number of 4 cysteines, contained carbo-

-hydrate and was found to have the N-terminal sequence Val-Asx-Asx-Lys-Thr-Phe-Ser-Val-.

Prior to chymotryptic digestion (on 740 nmol) the Fc"2 fragment previously radiolabelled with ^{14}C-iodo-acetate after partial reduction was subjected to total reduction and alkylated with ^{3}H-iodoacetic acid.

Nine chymotryptic peptides were isolated and their composition is shown in Table 17.

To produce overlapping peptides, 330 nanomoles of partially reduced-^{14}C-alkylated Fc"2 were digested with thermolysin for 16 hours. Only two peptides (TL)N1F1 and (TL)N1H1 provided valuable information and their composition is shown in Table 17. End group analysis of peptide (TL)N1F1 revealed the presence of two N-terminal residues: tyrosine and leucine, which indicated that (TL)N1F1 represented a mixture of two peptides. This was further confirmed by the sequence analysis, as two residues could be identified also after the first Edman degradation step. The following steps revealed a single residue after each degradation which indicated that (TL)N1F1 consisted of two peptides of different size. In view of the fact that (TL)N1F1 was not radioactive it was concluded that the cysteine residues identified were derived from an intra-chain disulphide bridge. By comparison with the known sequences around the cysteines determined in the CNBr-fragments of the epsilon chain the peptide (TL)N1F1 was assumed to have the following structure:

Leu-Cys Tyr-Thr-Cys-Glx

The constituent peptides in (TL)N1F1 would thus give an overlapping sequence between (C)A4F and (C)A5E as well as between (C)N6C and (C)A2C and in addition indicate the presence of an intra-chain disulphide bridge in Fc"2. Peptide (TL)N1H1 represented a pentapeptide with the sequence Tyr-Thr-Pro-Gly-Thr , which gave an overlap between the chymotryptic peptides (C)A5E and (C)A4B1.

Tryptic cleavage of Fc"2 (400 nmol) yielded 9 peptides but only a large core peptide characterized by the N-terminal sequence Ile-Leu-Glx- and a peptide (T)N6B2 lacking both lysine and arginine were subjected to further analysis (Table 17). After purification by HVE at pH 6.5 the core peptide was digested in situ with pepsin for 16 hours. Of the several peptides separated by reelectrophoresis at pH 6.5 only one peptide (TP)A3A1: A2 was further studied. The sequence of this tetradecapeptide was found to represent a segment that runs from the C-terminal end of peptide (C)A4B1 to the third aminoterminal residue in (C)A1B. The other tryptic peptide (T)N6B2 was found to have the sequence Gly-Val-Ser-Ala , which was assumed to represent the C-terminal end of Fc"2. Comparison with the C-terminal sequence of the peptide (C)A4A from CNBr-fragment G(III) (Fig. 11) gave support to the conclusion that (T)N6B2 represents the C-terminal sequence of Fc"2.

The complete amino acid sequence of Fc"2 is shown in Fig. 12 which also illustrates that the fragment overlaps the CNBr-fragments E(Fd´c) and G(III).

The location of acids and amides was determined as described above (E(Fd´c) and G(III) and in Table 7 and 14). Fc"2 contains 2 tryptophan residues, one located in (C)A4B1 the other in (C)B1D. Two oligosaccharide chains - CHO:C and CHO:D - were present in Fc"2. The side chain CHO:C, identified in the E(Fd´c) peptide (C)M1:1, was linked to an (presumably) asparaginyl residue at position 3 from the N-terminus and CHO:D was linked to an (presumably) asparaginyl residue in the sequence -Ile-Asn-Ile-Thr- of the peptide (C)A4B1. Their monosaccharide composition is shown in Table 6. The disposition of the peptides Fc"2, Fc"3 and Fc"4 in fragment Fc" is illustrated in Fig. 13 and will be further discussed below.

Alignment of CNBr-fragments

Isolation and structure of CM-methionine containing peptides. In order to identify overlapping sequences between the CNBr-fragments, IgE, (180 nmol) that had been treated with ^{14}C-iodoacetic acid under conditions favouring the S-carboxymethylation of methionine, was digested with trypsin. Four radioactive peptides - A, B1, B2 and N2 were identified and further purified and their amino acid composition is shown in Table 18. Due to the conversion of CM-methionine to homoserine-homoserine lactone, methionine and carboxymethylhomocysteine that takes place during hydrolysis, the recovery was not calculated except for methionine.

Sequence analysis of the peptides showed that peptide A (Asx-Ala-Ser-Phe-Ser-Thr-Ala-Tyr-Met-Asx-Leu-Arg) represented an overlapping sequence between CNBr-fragments D(VI) and F(Fd´a), while peptide B1 (Val-Thr-Met-Thr-Arg) represented an overlapping segment between the H(Fd´b) and D(VI) fragments.

The peptide B2 was found to be a mixture of 3 peptides present in the proportions 1:1:0.2 as shown in Table 18. Fortunately, as further purification failed, the sequence could be determined in parallel for all three peptides from the analysis of B2. The structure of B2:1 (Ala-Leu-Met-Arg) represented the overlap between CNBr-fragments G(III) and B(ε4-ε2), whereas the sequences Ala-Val-Ser-Val-Asn-Pro-Gly-Lys and Thr-Ser-Gly-Pro-Arg were derived from the C-terminal portion of the heavy chain as they are identical to the previously reported peptides ε2(T)B4B and ε4(T)B3 (Bennich et al., 1973). The structure of the peptide N2 was determined to be Leu-Met-Ile-Tyr-Glx-Val-Thr-Lys . As this sequence was not found in any of the CNBr-fragments from the epsilon chain ND, it must be derived from the lambda chain ND, which is known to contain one methionine residue (Bennich & Johansson, 1971) presumably located in the variable region.

From the analysis of radiolabelled methionine peptides, the order of the CNBr-fragments could be determined to be H(Fd´b)-D(VI)-F(Fd´a) and G(III)-B(ε4-ε2).

As sequence analysis of Fc"2 revealed a linkage

between E(Fd´c) and G(III) as described above, fragment C(V) must be located between F(Fd´a) and E(Fd´c) which completes the order of all CNBr fragments in the epsilon chain ND as follows: H(Fd´b)-D(VI)-F(Fd´a)-C(V)-E(Fd´c)-G(III)-B(ε4-ε2).

Though the recovery of 8 CNBr fragments is in agreement with the presence of 7 methionine residues in the epsilon chain ND as calculated from the amino acid data it cannot be excluded that a small methionine peptide has been lost. The location of such a missing CNBr-fragment must be either between F(Fd´a) and C(V) or between C(V) and E(Fd´c). The possibility of a missing CNBr-peptide in fragment B could be excluded by the existence of a peptic peptide released during the conversion of IgE to $F(ab')_2$ with sequence: Ile-Glx-Asx-Phe-Met-Pro-Glx-Asx , which proves the linkage between ε4 and ε2.

Determination of inter- and intra-chain disulphide bridges

The amino acid composition of the epsilon chain ND indicated the presence of 15 cysteine residues for a polypeptide per Mw 62 000. The assignment of three half-cystines to inter-chain bonds and 12 to the formation of intra-chain bridges was determined as follows as it is known that the inter-chain disulphide bonds are reduced first (Cecil & Wake, 1962): An analysis of the distribution of radiolabelled CNBr-fragments from partially reduced-^{14}C-alkylated and totally reduced-^{14}C-alkylated epsilon chain ND revealed that

fragments H(Fd′b) and B(ε4-ε2) contained intra-chain half-cystines, whereas the fragments E(Fd′c), F(Fd′a) and G(III) each contained four half-cystine residues involved in both inter- and intra-chain bridges.

The location of radiolabelled half-cystines in the fragments isolated from a partially reduced-^{14}C-alkylated digest (see METHODS) was as follows:

E(Fd′c)	Phe-Ser-Val-Cys-Ser-Arg	(cf (C)N5, Fig. 7)
E(Fd′c)	Glx-Ser-Ser-Cys-Asx-Gly	(cf (C)A3D, Fig. 7)
F(Fd′a)	-Cys-Cys-Lys	(cf (T)A3A2, Fig. 8)
G(III)	Thr-Lys-Lys-Cys-Ala-Asp	(cf (C)A4A, Fig. 11)

These results indicate that 5 of the 15 half-cystines in the epsilon chain ND might represent inter-chain bonds of which one links the lambda and the epsilon chain leaving 4 to be inter-epsilon-chain bonds.

However, different results indicating only two inter-epsilon-chain bonds were obtained by a study of the Fc" fragment.

As described previously Fc" consists of three disulphide linked peptides, of which two (Fc"2 and Fc"3) are derived from the epsilon chain and one (Fc"4) represents the C-terminal sequence of the lambda chain.

Double labelling of the half-cystines in Fc" using ^{14}C- and ^{3}H-iodoacetate after partial and total reduction respectively revealed the presence of three groups of disulphide bonds with different ^{3}H/^{14}C ratio as shown in Table 19.

A high ^{3}H/^{14}C ratio (70-80) expected to be given by an intra-chain linked half-cystine residue was obtained

for the two half-cystines forming the intra-chain disulphide bridge between peptides (C)A5E and (C)A2C in Fc"2 as described above.

A low $^{3}H/^{14}C$ ratio expected to be given by an inter-(labile) chain disulphide bond was obtained for three half-cystines of which two were present in Fc"3 and one in Fc"2. As the inter-light-heavy chain bonds in IgE are known to be cleaved by mild reduction (Johansson & Bennich, 1967) one of the labile bonds found must represent the lambda-epsilon chain bridge, which thus could be located in either Fc"2 or Fc"3. However, as the peptides Fc"2 and Fc"3 are disulphide linked in Fc", it could be concluded that the lambda chain must be linked to one of the half-cystines in Fc"3.

Two half-cystines residues located in Fc"2 were found to have an "intermediate" $^{3}H/^{14}C$ ratio of 3-6. As Fc" must represent a covalently linked dimer it was concluded that these half-cystines must represent the inter-epsilon-chain bonds.

The diagrammatic structure of Fc" can thus be illustrated as shown in Fig. 13. Further if it is assumed that five of the intra-chain bonds in the epsilon chain are linked in homology with the intra-chain bonds in the gamma- and mu-chains the results of the present study can be summarized as shown in Fig. 14 and Fig. 15 and Table 20. As shown in Table 20 the amino acid composition obtained by amino acid analysis differs from that based on the sequence data by $\pm$ 15 residues. Though this figure is close to the analytical error

(2 %) we cannot exclude that some residues presumably in the peptide not yet sequenced have not been accounted for.

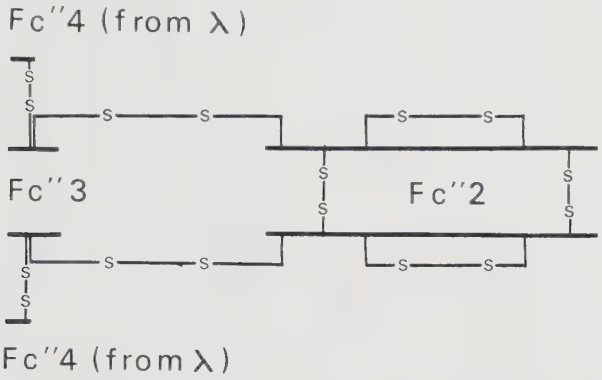


FIG. 13. Diagrammatic structure of Fc"-ε showing the disulphide bridges between the three polypeptide chains, Fc"2, Fc"3 and Fc"4.

FIG. 6 . PRIMARY STRUCTURE OF FRAGMENT V. Tryptic (T), chymotryptic (C) and peptic (P) peptides were sequenced by the dansyl-Edman method (⟶) and by use of carboxypeptidase B (⟵). Unidentified residues are indicated by (--⟶) carbohydrate side chain is denoted CHO.

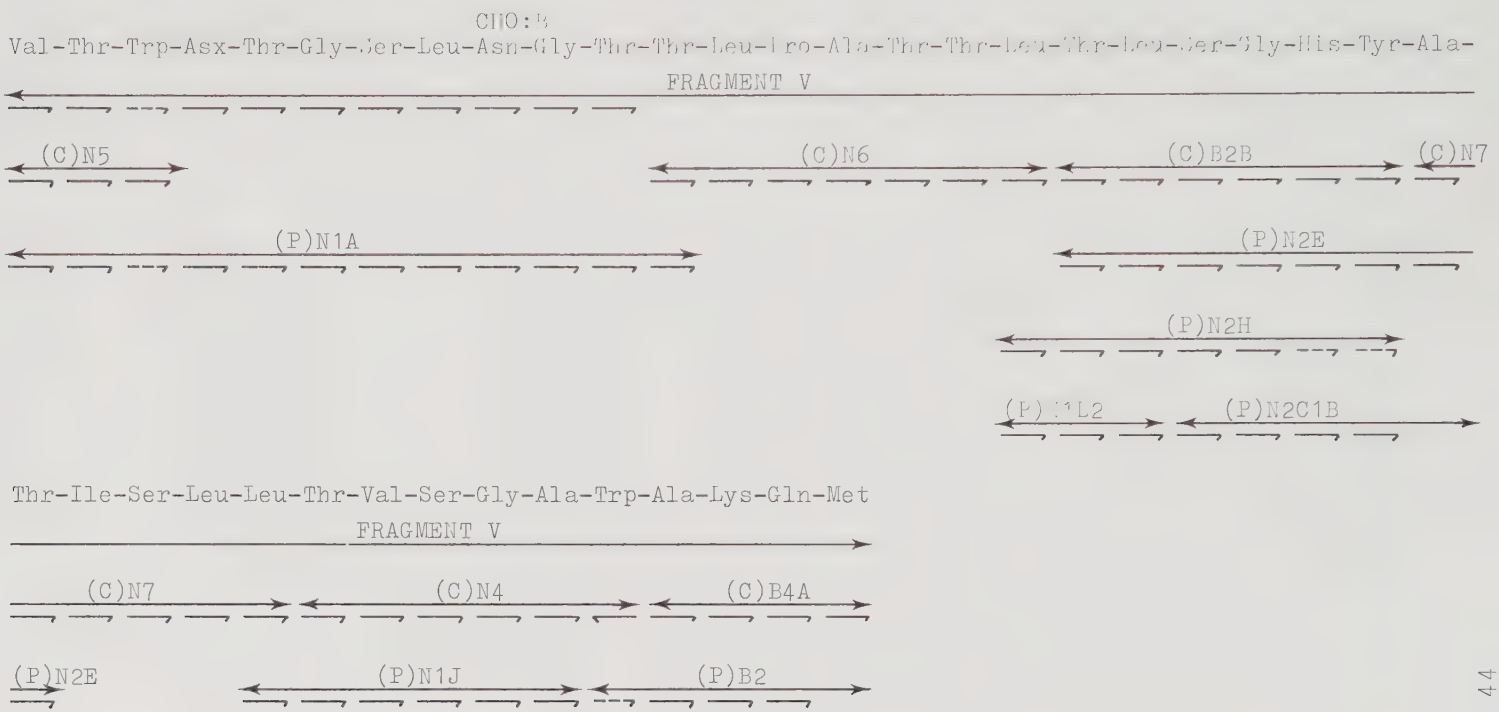


FIG. 7 . PRIMARY STRUCTURE OF FRAGMENT Fd´c. Tryptic (T), chymotryptic (C) and thermolytic (TL) peptides were sequenced by the dansyl-Edman method (⟶), unidentified residues are marked (--→). The location of a carbohydrate side chain is denoted CHO. The C-terminal aspartic acid residue is not present in Fd´c but represents the C-terminus of peptide Fc"2(C)A4B1.

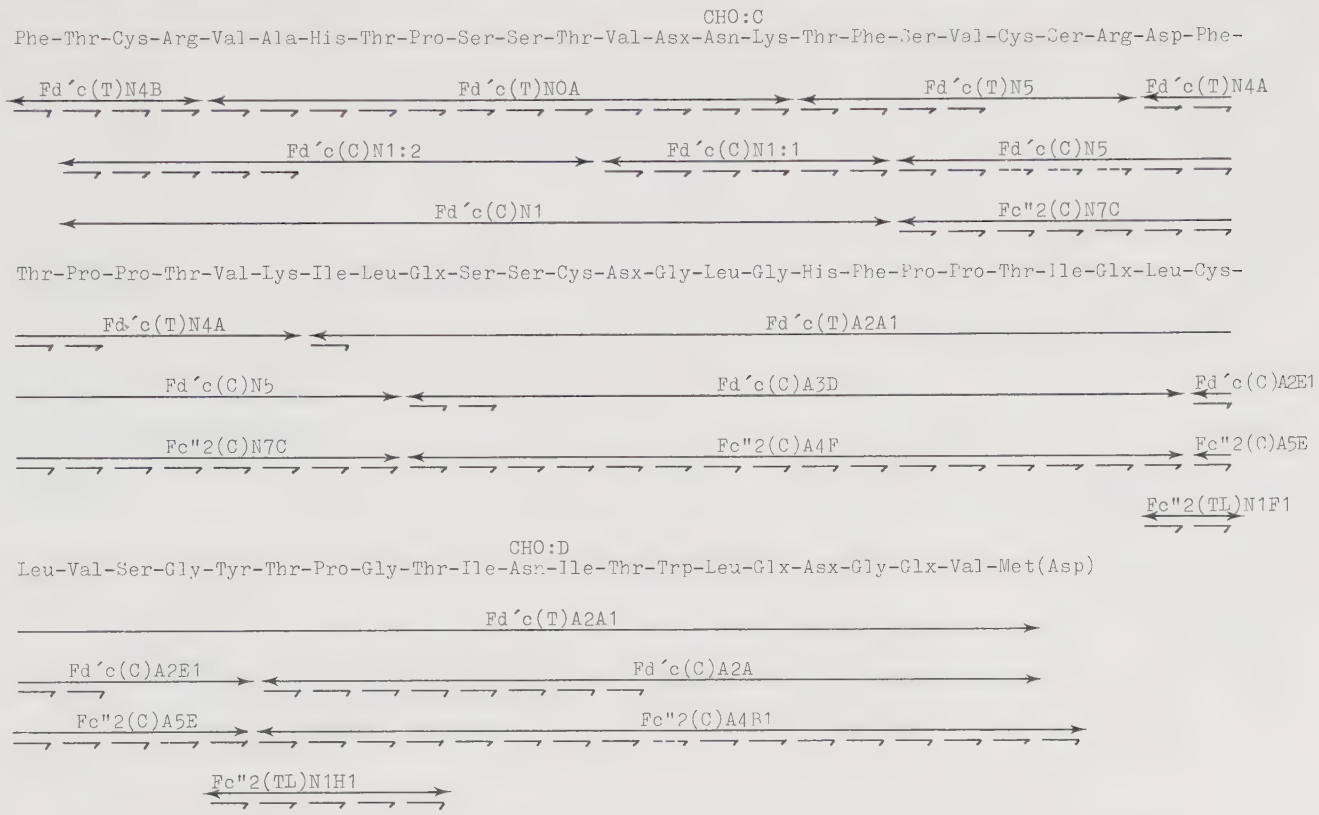


FIG. 8. PRIMARY STRUCTURE OF FRAGMENT Fd´a. Tryptic (T) and chymotryptic (C) peptides were sequenced by the dansyl-Edman method (——). Unidentified residues are in parenthesis. The location of a carbohydrate side chain is denoted CHO.

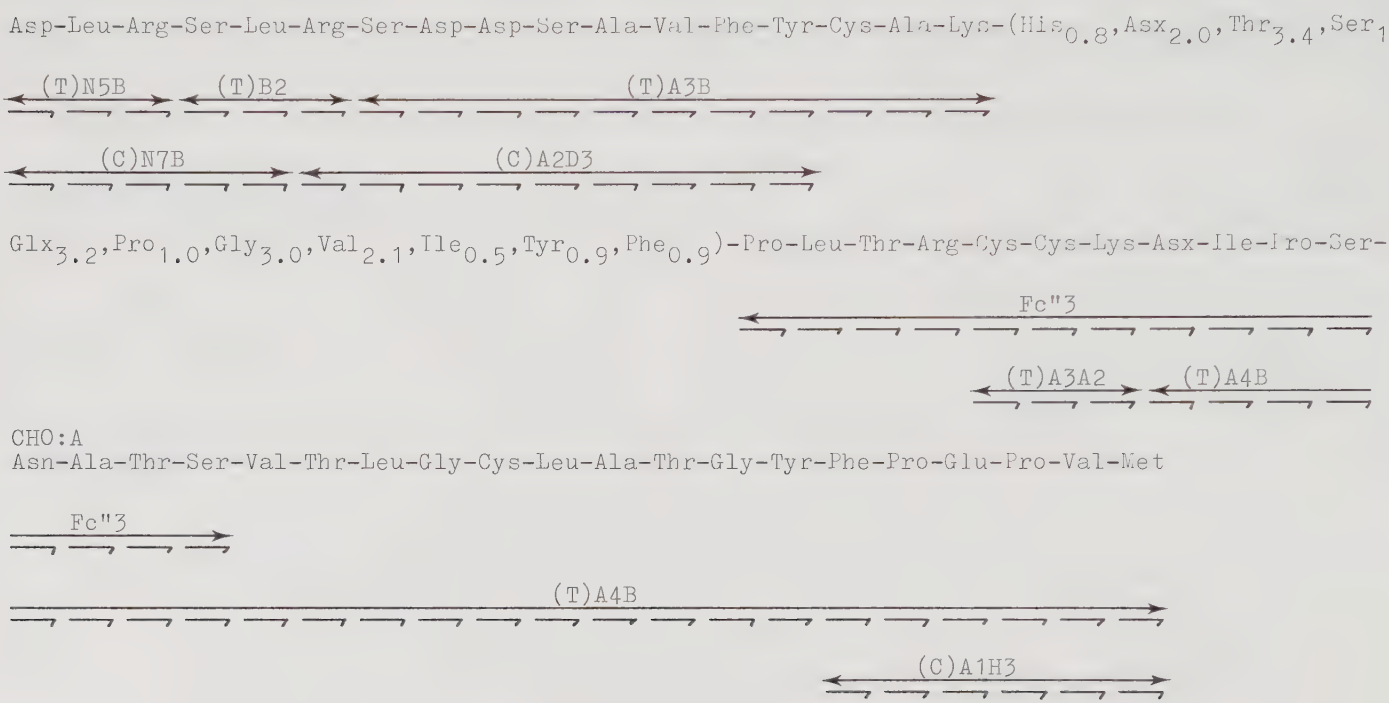


FIG. 9 . PRIMARY STRUCTURE OF FRAGMENT Fd´b. Tryptic (T), chymotryptic (C) and thermolytic (TL) peptides were sequenced by the dansyl-Edman method (⟶) and by use of carboxypeptidase B (⟵). Unidentified residues are in parenthesis or indicated by (⇢).

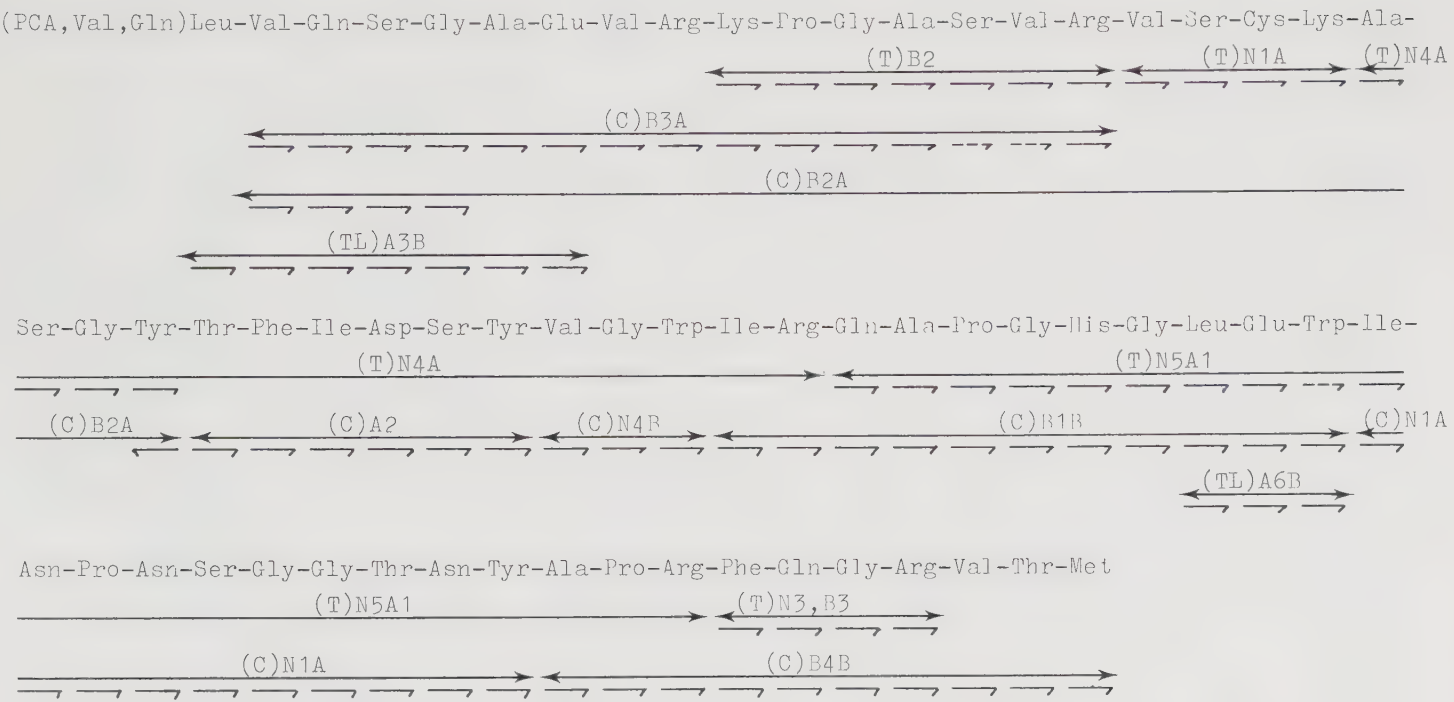
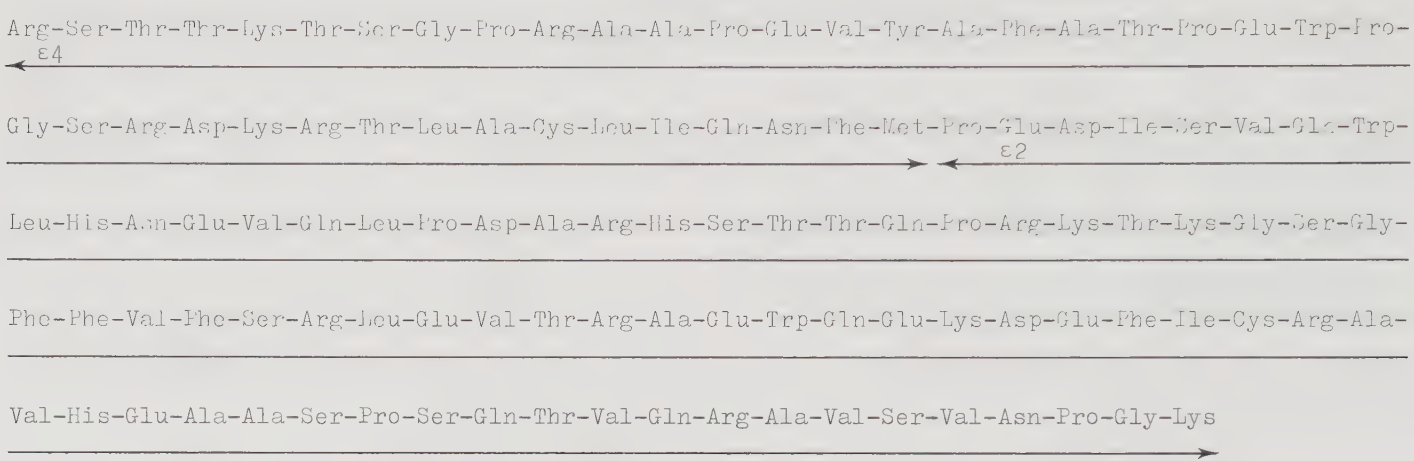


FIG. 10. PRIMARY STRUCTURE OF CHPr FRAGMENT P AND ITS CONSTITUENT FRAGMENTS ε4 AND ε2.[a)]



a) from Bennich et.al 1973.

G. 11 . PRIMARY SEQUENCE OF FRAGMENT III. Tryptic (T), chymotryptic (C), peptic (P), peptic-tryptic (TP) and thermolytic (TL) peptides were sequenced by the dansyl-Edman method (⟶) and by use of carboxypeptidase B (⟵). Double arrows indicate residues determined without hydrolysis, unidentified residues are indicated (--→). Ambigous acids and amides are underlined. Carbohydrate side chains are denoted CHO.

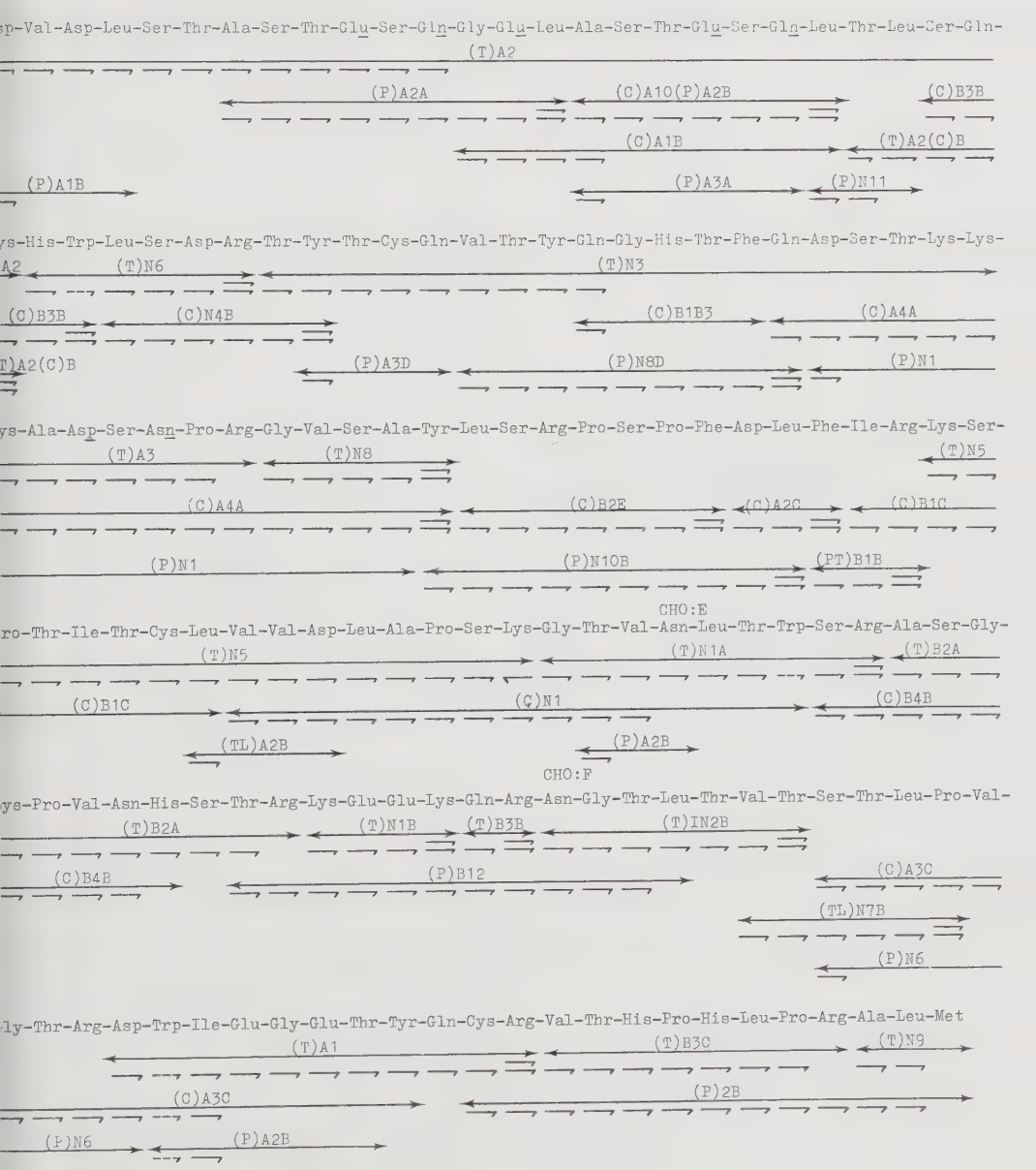
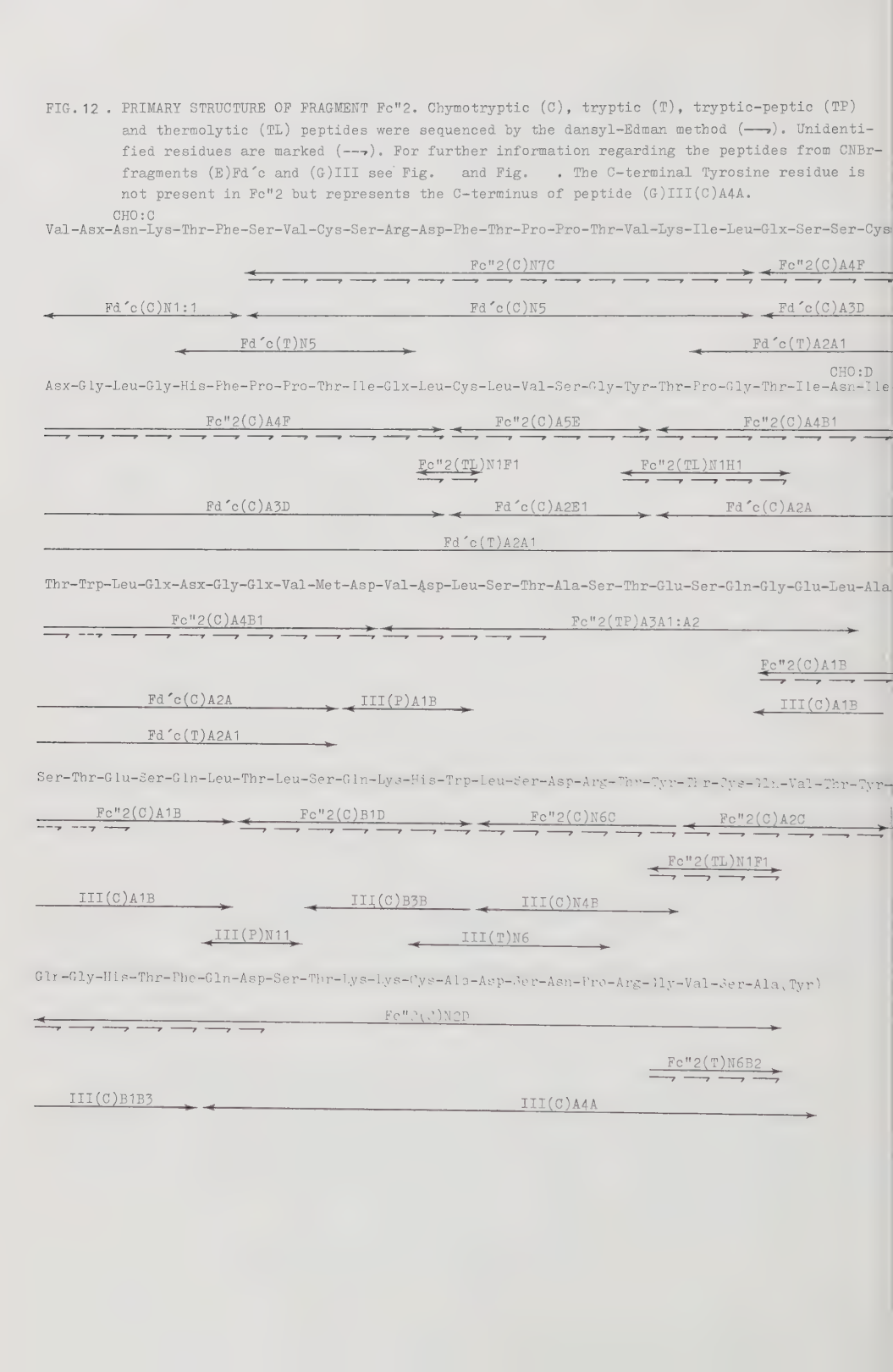


FIG. 12 . PRIMARY STRUCTURE OF FRAGMENT Fc"2. Chymotryptic (C), tryptic (T), tryptic-peptic (TP) and thermolytic (TL) peptides were sequenced by the dansyl-Edman method (⟶). Unidentified residues are marked (--→). For further information regarding the peptides from CNBr-fragments (E)Fd´c and (G)III see Fig. and Fig. . The C-terminal Tyrosine residue is not present in Fc"2 but represents the C-terminus of peptide (G)III(C)A4A.



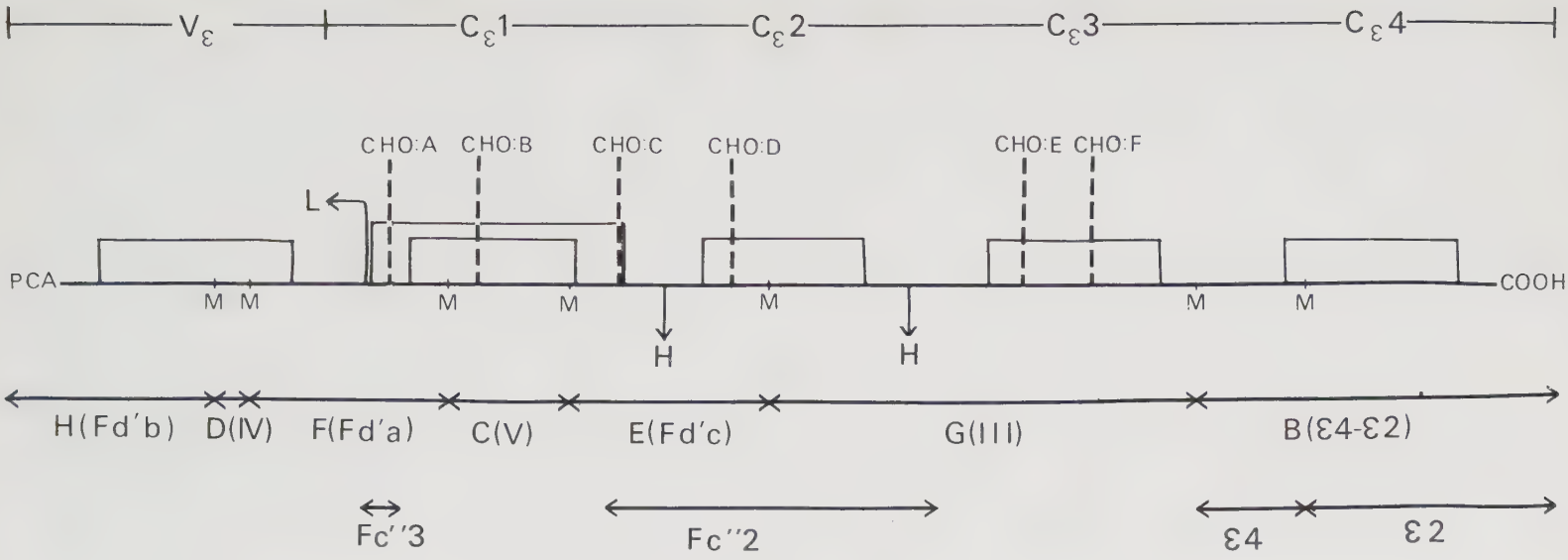


FIG.14. Diagrammatic structure of the ε(ND)-chain showing the location of CNBr-fragments, Fc"2 and Fc"3. M = methionine residues, CHO:A-F = carbohydrate side chains, H denotes an inter-heavy disulphide bond, and L an heavy-light disulphide bond.

FIG.15. PRIMARY STRUCTURE OF THE EPSILON CHAIN ND. Underlined asparagine denotes position of carbohydrate side chains.

TABLE 1. AMINO ACID COMPOSITION OF FRAGMENT V AND ITS CHYMOTRYPTIC (C) AND PEPTIC (P) PEPTIDES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fragment V	(C)N6	(C)B2B	(C)N7	(C)N4	(C)B4A	(C)N5	(P)N1A	(P)N2H	(P)N2E	(P)N1L2	(P)N2C1P	(P)N1J	(P)B2
Lys	1.1(1)					1.0(1)								
His	1.1(1)		1.0(1)						1.0(1)	0.9(1)		0.9(1)		
Asp	3.0(2)							1.7(2)						
Thr	8.9(9)	3.1(3)	0.8(1)	1.0(1)	0.9(1)		1.1(1)	4.1(4)	1.0(1)	1.7(2)	1.0(1)	1.0(1)	1.2(1)	
Ser	4.4(4)		1.2(1)	1.0(1)	1.1(1)			1.2(1)	1.0(1)	1.1(1)	0.5	1.0(1)	1.0(1)	
Homoser	0.7(1)					0.4(1)			0.3					0.4(1)
Glu	1.8(1)					1.1(1)			0.3					1.2(1)
Pro	1.3(1)	1.1(1)												
Gly	4.5(4)		1.2(1)		1.1(1)		0.5	2.0(2)	0.8(1)	1.1(1)		1.1(1)	1.0(1)	0.6
Ala	3.7(4)	0.9(1)		0.8(1)	1.0(1)	1.0(1)			0.6	1.0(1)		1.2(1)	1.1(1)	1.0(1)
Val	2.7(2)				1.0(1)		0.9(1)	0.8(1)					0.9(1)	
Ile	1.0(1)			1.1(1)					0.6		0.4			
Leu	6.4(6)	2.5(2)	1.1(1)	2.4(2)			0.1	1.3(1)	1.5(2)	1.1(1)	2.4(2)		0.9(1)	
Tyr	0.9(1)		0.9(1)						0.8(1)	0.8(1)		0.8(1)		
Phe									0.2					
CHO[a]	+							+						
Total residues	(38)	(7)	(6)	(6)	(5)	(4)	(2)	(11)	(7)	(8)	(3)	(6)	(6)	(3)
Yield														
nmol		56	68	45	66	87	112							
percent		10	12	8	11	15	19							

[a] detected on amino acid analysis

TABLE 2 . CARBOHYDRATE COMPOSITION OF OLIGOSACCHARIDE CHAIN B CONTAINING GLYCOPEPTIDES. Monosaccharides determined by gas chromatography are expressed as residues per mole of peptide.

Monosaccharide	V(P)N1A		Mean value
Fucose	1.2	1.7	1.5
Mannose	3.8	3.8	3.8
Galactose	2.7	2.6	2.7
N-acetyl glucosamine	5.1	4.9	5.0
Sialic acid	0.8	0.5	0.7

TABLE 3 . DETERMINATION OF ACIDS AND AMIDES IN FRAGMENT V.

Peptide	Mw	m	e	Amino acids contributing to charge Glx	Lys	Determined ac and amides
(C)B4A	440	0.49	+1	1	1	1Gln

TABLE 4 . AMINO ACID COMPOSITION OF FRAGMENT VI. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues, found by sequence analysis is shown in parenthesis.

	fragment VI
Arg	1.0(1)
Asp	1.0(1)
Thr	1.7(2)
Ser	2.0(2)
Homoser	0.5(1)
Ala	2.3(2)
Tyr	0.8(1)
Phe	1.2(1)
Total residues	(11)

TABLE 5. AMINO ACID COMPOSITION OF FRAGMENT Fd′c AND ITS TRYPTIC (T) AND CHYMOTRYPTIC (C) PEPTIDES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fragment Fd′c	(T)N4B	(T)NOA	(T)N5	(T)N4A	(T)A2A1	(C)N1	(C)N1:1[b]	(C)N1:2[b]	(C)N5	(C)A3D	(C)A3E1	(C)A2A
Lys	2.4(2)		0.9(1)		1.0(1)		1.6	1.0(1)		0.9(1)			
His	1.1(2)		0.9(1)			0.7(1)	1.0		1.0(1)		1.0(1)		
Arg	2.0(2)	1.0(1)		0.7(1)			+		+ (1)	1.1(1)			
CMCys	3.0(4)	1.2(1)		0.9(1)		1.2(2)	+		+ (1)	+ (1)	+ (1)	0.8(1)	
Asp	5.9(6)		2.3(2)		0.9(1)	2.9(3)	4.2	2.6(2)		1.1(1)	1.3(1)		2.0(2)
Thr	9.0(10)	0.9(1)	1.7(2)	1.0(1)	2.0(2)	4.0(4)	4.3	1.0(1)	2.7(3)	1.6(2)	1.0(1)		3.2(3)
Ser	8.3(7)		1.7(2)	1.8(2)		2.9(3)	2.5		2.5(2)	2.0(2)	2.0(2)	0.8(1)	
Homoser	+ (1)					0.6(1)							0.4(1)
Glu	5.6(4)					4.4(4)				0.6	2.3(2)		2.5(2)
Pro	7.7(6)		1.0(1)		2.2(2)	2.7(3)	+		+ (1)	1.7(2)	2.3(2)		+ (1)
Gly	5.1(5)					5.7(5)	0.9			0.9	3.7(2)	1.2(1)	2.0(2)
Ala	2.9(1)		1.0(1)				1.6		1.6(1)				
Val	4.8(6)		1.9(2)	1.0(1)	1.0(1)	2.4(2)	2.3	0.9(1)	0.9(1)	1.4(2)		1.0(1)	1.0(1)
Ile	2.3(4)					3.2(4)				0.8(1)	1.0(1)		1.6(2)
Leu	4.7(5)					4.4(5)				1.1(1)	2.2(2)	1.0(1)	1.3(1)
Tyr	2.0(1)					1.4(1)						0.7(1)	
Phe	3.7(4)	1.0(1)		0.9(1)	1.1(1)	0.9(1)	1.7	1.1(1)		0.8(1)	0.9(1)		
CHO[a]	+					+							
Total residues	(70)	(4)	(12)	(7)	(8)	(39)		(6)	(10)	(15)	(16)	(6)	(15)
Yield nmol		80	70	110	105	60							
percent		28	25	39	37	21							

[a] detected on amino acid analysis

[b] calculated composition

TABLE 6 . CARBOHYDRATE COMPOSITION OF OLIGOSACCHARIDE CHAIN C AND D CONTAINING GLYCOPEPTIDES. Monosaccharides determined by gas chromatography are expressed as residues per mole of peptide.

	CHAIN C					CHAIN D		
Monosaccharide	Fd´c(T)NOA				Mean value	Fd´c(C)A2A		Mean value
Fucose	trace	trace	trace	trace	trace	trace	trace	trace
Mannose	3.5	2.5	3.4	3.6	3.3	3.1	2.8	3.0
Galactose	2.5	2.6	2.4	2.8	2.6	3.0	2.2	2.6
N-acetyl-glucosamine	5.2	2.3	3.6	4.0	3.8	3.0	3.4	3.2
Sialic acid	-	2.8	2.4	1.7	2.3	1.1	1.4	1.3

TABLE 7 . DETERMINATION OF ACIDS AND AMIDES IN FRAGMENT Fd´c.

Peptide	Mw	m	e	Amino acids contributing to charge Asx	Glx	Lys	His	CMCys	Determined acids and amides
(T)N4A	880	0	0	1		1			1Asp
(C)A3D	1760	0.22	-1(-2)	1	2		1	1	1 acid 2 amides or 3 amides

TABLE 8 . AMINO ACID COMPOSITION OF FRAGMENT Fd´a AND ITS TRYPTIC (T) AND CHYMOTRYPTIC (C) PEPTIDES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fragment Fd´a	(T)N5B	(T)B2	(T)A3B	(T)A3A2	(T)A4B	(C)N7B	(C)A2D3	(C)A1H3
Lys	2.0(2)			1.2(1)	1.0(1)				
His	0.8								
Arg	2.5(3)	1.3(1)	0.9(1)				1.0(1)	1.0(1)	
CMCys	2.0(4)			0.9(1)	2.0(2)	0.9(1)			
Asp	7.0(5)	1.0(1)		2.2(2)		2.1(2)	1.0(1)	2.0(2)	
Thr	7.4(4)					2.7(3)			
Ser	6.7(5)		1.1(1)	1.8(2)		2.3(2)	1.0(1)	1.9(2)	
Homoser	0.6(1)					0.6(1)			+ (1)
Glu	4.2(1)					1.3(1)			1.0(1)
Pro	5.0(4)					2.4(3)			1.8(2)
Gly	5.0(?)					2.0(?)			
Ala	4.2(4)			2.5(2)		1.9(2)		1.2(1)	
Val	5.1(3)			1.1(1)		1.9(2)		1.5(1)	1.0(1)
Met	0.2								
Ile	1.5(1)					1.0(1)			
Leu	5.1(5)	1.0(1)	1.0(1)			2.0(?)	1.9(?)		
Tyr	2.9(?)			1.0(1)		0.8(1)		0.9(1)	
Phe	2.9(?)			1.0(1)		1.0(1)		0.9(1)	+ (1)
CHO[a]	+					+			
Total residues		(3)	(3)	(11)	(3)	(24)	(5)	(9)	(6)
Yield nmol		242	149	85	240	264			
Yield percent		24	15	9	24	26			

TABLE 9. CARBOHYDRATE COMPOSITION OF OLIGOSACCHARIDE CHAIN A CONTAINING GLYCOPEPTIDES. Monosaccharides determined by gas chromatography are expressed as residues per mole of peptide.

Monosaccharide	Fc"3	Mean value
Fucose	1.2 1.3	1.3
Mannose	3.2 3.3	3.3
Galactose	2.5 2.5	2.5
N-acetyl-glucosamine	3.1 3.9	3.9
Sialic acid	0.4 0.5	0.5

TABLE 10. DETERMINATION OF ACIDS AND AMIDES IN FRAGMENT Fd´a.

Peptide	Mw	m	e	Amino acids contributing to charge Asx	Glx	Arg	Determined acids and amides
(C)N7B	550	0	0	1		1	Asp
(C)A2D3	990	0.44	-1	2		1	2Asp
(C)A1N3	660	0.44	-1		1		Glu

TABLE 11 . AMINO ACID COMPOSITION OF THE FRAGMENT Fd´b AND ITS TRYPTIC (T) AND CHYMOTRYPTIC (C) PEPTIDES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fragment Fd´b	(T)B2	(T)N1A	(T)N5A1	(T)N3	(C)B3A	(C)B2A	(C)A2	(C)N4B	(C)B1B	(C)N1A	(C)B4B	(TL)A3B	(TL)A6B
Lys	2.3(2)	1.0(1)	1.1(1)			1.0(1)	2.0(2)							
His	1.6(1)			0.5(1)						1.1(1)				
Arg	4.0(5)	1.0(1)		0.7(1)	1.0(1)	1.9(2)	1.9(2)			0.9(1)		2.1(2)		
CMCys	1.0(1)		0.7(1)				0.4(1)							
Asp	4.2(4)			2.9(3)				1.1(1)			3.0(3)			
Thr	4.8(3)			1.0(1)				1.0(1)			1.1(1)	1.0(1)		
Ser	6.8(6)	0.9(1)	1.0(1)	1.2(1)		1.9(2)	3.8(4)	1.0(1)			1.1(1)		1.0(1)	
Homoser	0.4(1)											0.5(1)		
Glu	7.0(7)			2.1(2)	1.0(1)	2.2(2)	2.0(2)			2.0(2)		1.1(1)	2.1(2)	1.0(1)
Pro	2.9(4)	0.9(1)		3.2(3)		+ (1)	1.0(1)			0.6(1)	1.1(1)	1.2(1)		
Gly	7.8(9)	1.1(1)		4.3(4)	1.0(1)	2.2(2)	3.2(3)		0.9(1)	2.2(2)	2.1(2)	1.1(1)	1.1(1)	
Ala	5.1(5)	1.1(1)		1.8(2)		2.1(2)	3.1(3)			1.1(1)		1.0(1)	0.9(1)	
Cys							0.4							
Val	4.5(7)	0.9(1)	1.0(1)	1.1		2.9(3)	3.7(4)		1.1(1)			1.0(1)	0.7(1)	
Ile	2.8(3)			0.8(1)				1.0(1)		0.9(1)	0.9(1)			
Leu	2.3(2)			1.1(1)						1.2(1)			1.0(1)	1.0(1)
Tyr	2.7(3)			0.6(1)			0.7(1)	0.9(1)			0.8(1)			
Phe	1.9(2)				1.0(1)			1.1(1)				0.9(1)		
Total residues	(65)	(6)	(4)	(21)	(4)	(15)	(20)	(6)	(2)	(10)	(10)	(10)	(7)	(2)
Yield nmol		154	152	37	81									
percent		15	15	4	8									

TABLE 12. DETERMINATION OF ACIDS AND AMIDES IN FRAGMENT Fd´b

Peptide	Mw	m	e	Amino acids contributing to charge Asx	Glx	Arg	His	Determined acids and amides
(TL)A3B	770	0.33	-1		2			1Gln 1Glu
(C)A2	660	0.32	-1	1				1Asp
(C)B1	1100	0.14	+1	2		1	1	1Gln 1Glu
(TL)A6B	330	0.43	-1		1			1Gln
(C)N1A	1100	0	0	3				3Asn
(C)B4B	1100	0.49	+2		1	2		1Gln
(T)N3	440	0	0		1	1		1Glu
(T)B3	440	0.36	+1		1	1		1Gln

TABLE 13a. AMINO ACID COMPOSITION OF FRAGMENT III AND ITS TRYPTIC (T) PEPTIDES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fragment III	(T)A2	(T)N6	(T)N3	(T)A3	(T)N8	(T)N5	(T)N1A	(T)B2A	(T)N1B	(T)B3B	(T)IN2B	(T)A1	(T)B3C	(T)N9
Lys	8.0(8)	1.4(1)		3.0(2)			2.6(2)		1.2(1)	0.9(2)			0.2		
His	5.1(5)		0.6(1)	1.5(1)					0.9(1)					2.1(2)	
Arg	9.9(10)		0.8(1)		0.9(1)			0.9(1)	1.1(1)		0.9(1)		0.7(1)	1.0(1)	
CMCys	3.4(4)			0.7(1)	1.0(1)		0.6(1)						1.0(1)		
Asp	11.7(12)	1.8(2)	1.0(1)	1.0(1)	2.0(2)		1.2(1)	1.0(1)	1.1(1)			1.3(1)	1.0(1)		
Thr	20.8(21)	3.6(4)		4.0(5)			2.0(2)	2.0(2)	1.0(1)			2.7(3)	1.0(1)	0.9(1)	
Ser	17.0(18)	5.6(6)	1.1(1)	1.7(1)	1.0(1)	0.9(1)	3.0(2)	1.2(1)	2.0(2)				0.5		
Homoser	n d[b](1)														0.4(1)
Glu	14.5(15)	6.4(6)		2.9(3)						1.0(2)	1.0(1)		2.7(3)		
Pro	10.0(9)				0.9(1)		2.0(2)		1.0(1)					0.9(1)	
Gly	8.7(8)	1.6(1)		0.8(1)		1.0(1)	0.2	1.1(1)	1.2(1)			1.0(1)	1.0(1)		
Ala	7.8(7)	1.8(2)			0.9(1)	1.1(1)	1.1(1)		1.2(1)						1.0(1)
Cys	1.8														
Val	10.0(10)	0.9(1)		1.0(1)		1.0(1)	1.6(2)	1.2(1)	1.1(1)			0.7(1)		1.0(1)	
Met	trace														
Ile	3.1(3)						0.8(1)						0.7(1)		
Leu	13.4(14)	3.0(4)	1.1(1)				1.8(2)	1.0(1)				0.9(1)	0.2	1.1(1)	1.0(1)
Tyr	4.1(4)			2.2(2)		1.0(1)							+ (1)		
Phe	3.5(3)			0.8(1)											
CHO[a]	+							+				+			
Total residues	(152)	(27)	(5)	(18)	(7)	(5)	(16)	(8)	(11)	(4)	(2)	(7)	(10)	(7)	(3)
Yield															
nmol		84	63	32	164	58	52	31	116	160	42	60	32	57	94
percent		17	13	6	33	12	10	6	23	32	8	12	6	11	19

[a] detected on amino acid analysis

[b] not determined

TABLE 13b. AMINO ACID COMPOSITION OF THE CHYMOTRYPTIC (C) PEPTIDES OF FRAGMENT III. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues, found by sequence analysis, is show in parenthesis.

	(C)A1B	(T)A2(C)B	(C)B3B	(C)N4B	(C)B1B3	(C)A4A	(C)B2E	(C)A2C	(C)B1C	(C)N1	(C)B4B	(C)A3C
Lys		1.0(1)	0.9(1)		0.2	2.1(2)			0.8(1)	1.0(1)	1.2(1)	
His			0.9(1)		0.8(1)						1.0(1)	
Arg				0.9(1)	0.3	1.0(1)	1.0(1)		1.0(1)		1.1(1)	0.9(1)
CMCys						0.8(1)			0.6(1)			
Asp	0.4			0.9(1)	0.2	3.2(3)		1.0(1)		2.1(2)	1.0(1)	1.0(1)
Thr	1.1(1)	0.8(1)		1.1(1)	1.3(1)	1.2(1)			1.9(2)	1.7(2)		3.4(3)
Ser	1.8(2)	1.0(1)	0.8(1)	1.0(1)	0.4	3.0(3)	1.6(2)		1.5(1)	1.5(1)	1.8(2)	0.9(1)
Glu	2.6(3)	1.1(1)	1.2(1)		1.0(1)	1.1(1)				0.6		2.1(2)
Pro					0.4	1.2(1)	2.2(2)		1.5(1)	+ (1)	1.1(1)	1.1(1)
Gly	1.6(1)		0.4		1.0(1)	1.2(1)				1.5(1)	1.2(1)	1.9(2)
Ala	1.1(1)				0.3	2.3(2)				1.2(1)	0.9(1)	
Val						1.1(1)				1.8(3)	1.0(1)	1.6(1)
Ile									1.6(2)			0.9(1)
Leu	2.0(2)	1.0(1)		1.1(1)	0.3		1.0(1)	1.0(1)	1.1(1)	1.7(2)		1.0(1)
Tyr				1.0(1)		1.1(1)						0.9(1)
Phe					0.8(1)		1.0(1)	1.0(1)				
CHO[a]										+		
Total residues	(10)	(5)	(4)	(6)	(5)	(18)	(7)	(3)	(10)	(14)	(10)	(15)

[a]detected on amino acid analysis

TABLE 12c. AMINO ACID COMPOSITION OF THE PEPTIC (P), PEPTIC-TRYPTIC (PT) AND THERMOLYTIC (TL) PEPTIDES OF FRAGMENT III. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	(P)A1B	(P)A2A	(P)A3A	(C)A10(P)A2B	(P)N11	(P)A3D	(P)N8D	(P)N1	(P)N10B	(PT)B1B	(TL)A2B	(P)A2B	(P)B12	(TL)N7B	(P)N6	(P)A2B	(P)2B
Lys								2.2(2)					2.0(2)				0.4
His							1.0(1)										1.3(2)
Arg								1.0(1)	1.0(1)	0.9(1)			1.8(2)		0.8(1)		1.7(2)
CMCys						1.0(1)		0.6(1)									0.4(1)
Asp	1.8(2)							2.9(3)	1.0(1)		1.4(1)	1.1(1)	1.1(1)		1.2(1)		0.4
Thr		2.1(1)	0.9(1)	0.9(1)	1.0(1)	1.0(1)	2.0(2)	1.2(1)				0.9(1)	1.7(2)	2.1(2)	1.5(2)	0.9(1)	1.4(1)
Ser		1.4(2)	1.9(2)	1.8(2)				2.7(3)	1.8(2)					1.0(1)	1.2(1)		0.7
Homoser																	0.2(1)
Glu		3.3(3)	2.2(2)	2.1(1)		1.3(1)	2.2(2)						2.7(3)			2.3(2)	1.6
Pro								1.0(1)	2.0(2)					1.2(1)	1.2(1)		1.7(2)
Gly		1.1(1)					1.2(1)	1.1(1)					0.5(1)		1.2(1)	1.3(1)	0.5
Ala		0.9(1)	0.9(1)	0.9(1)				2.0(2)									1.0(1)
Val	1.1(1)						1.0(1)	1.0(1)			1.8(2)	1.0(1)		1.3(1)	1.2(1)		1.0(1)
Ile										1.0(1)						0.8(1)	
Leu	0.9(1)	1.0(1)		1.4(1)	2.1(2)				2.0(2)		1.0(1)		0.1(1)	1.0(1)	1.1(1)		1.7(2)
Tyr						1.1(1)	1.1(1)		0.8(1)								0.2
Phe							1.0(1)		1.1(1)	1.0(1)							
Total residues	(4)	(9)	(6)	(7)	(3)	(4)	(9)	(16)	(10)	(3)	(4)	(3)	(12)	(6)	(9)	(5)	(13)

TABLE 14. DETERMINATION OF ACIDS AND AMIDES IN FRAGMENT III.

Peptide	Mw	m	e	Amino acids contributing to charge Asx	Glx	Lys	Arg	His	CMCys	Determined acids and amides
(P)A1B	440	0.88	-2	2						2Asp
(P)A2A	990	0.58	-2		3					2Glu 1Gln
(P)A3A	660	0.41	-1		2					1Glu 1Gln
(T)A2C	440	0.36	+1		1	1				1Gln
(C)N4B	660	0	0	1			1			1Asp
(P)A3D	440	0.41	-1		1				1	1Gln
(P)N8D	880	0	0		2			1		2Gln
(C)B1B3	550	0.18	+1		1			1		1Gln
(P)N1	1760	0	0	3		2	1		1	2Asp 1Asn
(T)A3	770	0.36	-1	2			1		1	1Asp 1Asn
(C)A2C	330	0.67	-1	1						1Asp
(TL)A2B	440	0.88	-1	1						1Asp
(T)B2A	1210	0.39	+2	1		1	1	1		1Asn
(T)N1B	440	0	0		2	2				2Glu
(T)B3B	220	0.55	+1		1		1			1Gln
(T)A1	1210	0.62	-3	1	3		1		1	3 acids 1 amide
(P)A2B	770	0.58	-2		2					2 Glu
(P)N6	990	0	0	1			1			1Asp

TABLE 15. CARBOHYDRATE COMPOSITION OF OLIGOSACCHARIDE CHAIN E AND F CONTAINING GLYCOPEPTIDES. Monosaccharides determined by gas chromatography are expressed as residues per mole of peptide.

	CHAIN E					CHAIN F		
Monosaccharide	III(P)A2B				Mean value	III(P)B12		Mean value
Fucose	0.9	0.4	0.4	1.0	0.7	-	-	-
Mannose	4.4	3.1	2.7	3.6	3.4	5.6	5.7	5.7
Galactose	2.4	1.7	1.8	2.5	2.1	0.3	0.3	0.3
N-acetyl-glucosamine	3.4	3.3	3.3	3.1	3.3	1.5	1.8	1.7
Sialic acid	0.9	1.6	1.1	1.8	1.3	-	-	-

TABLE 16. AMINO ACID COMPOSITION OF FRAGMENT Fc" AND ITS CONSTITUENT PEPTIDES RELEASED BY REDUCTION. Compositions are expressed as amino acid residues per mol of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fc"	Fc"1	Fc"2	Fc"3	Fc"4
Lys	5.8(6)	5.0	5.3(5)	1.0(1)	
His	3.5(3)	3.7	2.9(3)		
Arg	3.5(4)	2.7	3.6(3)	1.0(1)	
CMCys		1.8	3.9[b](5)	2.0(2)	
Asp	14.0(14)	12.0	11.5(12)	2.1(2)	
Thr	21.2(20)	17.0	17.5(17)	2.4(2)	0.9(1)
Ser	17.6(18)	13.5	13.8(15)	2.0(2)	0.9(1)
Glu	14.6(14)	13.1	14.0(13)		1.4(1)
Pro	11.0(9)	6.6	9.6(6)	2.0(2)	0.7(1)
Gly	9.5(8)	9.1	11.2(8)		
Ala	4.5(5)	3.4	5.7(4)	1.0(1)	
Cys	7.1(8)[c]	0.8			1.0(1)[c]
Val	8.6(8)	7.6	6.9(8)		
Met	0.9(1)	1.0	1.0(1)		
Ile	4.8(5)	4.0	4.0(4)	0.9(1)	
Leu	11.3(11)	10.2	10.5(10)	1.0(1)	
Tyr	3.4(3)	3.0	3.4(3)		
Phe	3.7(4)	3.8	4.1(4)		
CHO[a]	+	+	+	+	
Total residues	(141)		(121)	(15)	(5)

[a] detected on amino acid analyses
[b] determined on totally reduced Fc"2
[c] as cysteic acid

TABLE 17. AMINO ACID COMPOSITION OF THE FRAGMENT Fc"2 AND ITS CHYMOTRYPTIC (C), THERMOLYTIC (TL), TRYPTIC (T) AND TRYPTIC-PEPTIC (TP) PEPTIDES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	Fragment Fc"2	(C)N7C	(C)A4F	(TL)N1F1	(C)A5E	(C)A4B1	(TL)N1H1	(TP)A3A1:A2	(C)A1B	(C)B1D	(C)N6C	(C)A2C	(C)N2D	(T)N6B2
Lys	5.3(5)	1.0(1)				0.5				1.0(1)			2.3(2)	
His	2.9(3)		0.9(1)							0.8(1)			1.0(1)	
Arg	3.6(3)	1.0(1)									1.0(1)		1.0(1)	
CMCys	3.9[b](5)	1.0(1)	0.8(1)		0.7(1)							1.1(1)	0.8(1)	
Asp	11.5(12)	1.1(1)	1.1(1)			3.3(3)		1.9(1)	0.4		0.9(1)		3.0(3)	
Thr	17.5(17)	1.9(2)	0.8(1)	0.9(1)		2.8(3)	2.1(2)	2.2(2)	1.1(1)	0.8(1)	0.9(1)	2.1(2)	2.0(2)	
Ser	13.8(15)	1.9(2)	1.7(2)	0.5	0.8(1)			2.5(3)	1.8(2)	0.9(1)	1.1(1)		3.2(3)	1.1(1)
Glu	14.0(13)		2.0(2)	1.3(1)		1.5(2)		3.0(3)	2.6(3)	0.8(1)		1.0(1)	1.4(2)	
Pro	9.6(6)	1.8(2)	1.8(2)			0.9(1)	1.0(1)						3.0(1)	
Gly	11.2(8)		3.4(2)	0.4	1.0(1)	1.5(2)	1.0(1)	2.2(1)	1.6(1)				1.8(2)	0.9(1)
Ala	5.7(4)							1.0(1)	1.1(1)				1.8(2)	0.9(1)
Cys				+ (1)										
Val	6.9(8)	2.2(2)			1.2(1)	1.7(1)		1.4(1)				1.1(1)	0.9(1)	1.1(1)
Met	1.0(1)					0.8(1)								
Ile	4.0(4)	1.0(1)	1.1(1)			1.6(2)								
Leu	10.5(10)	1.1(1)	2.3(2)	1.0(1)	1.1(1)	0.9(1)		3.0(2)	2.0(2)	1.2(1)	1.1(1)			
Tyr	3.4(3)			0.7(1)	1.0(1)		0.9(1)				0.9(1)	1.0(1)		
Phe	4.1(4)	1.1(1)	1.0(1)			0.5							0.8(1)	
CHO[a]	+					+								
Total residues	(121)	(15)	(16)	(5)	(6)	(16)	(5)	(14)	(10)	(6)	(6)	(6)	(22)	(4)

[a]detected on amino acid analysis [b]determined on totally reduced Fc"2

TABLE 18. AMINO ACID COMPOSITION OF THE PEPTIDES CONTAINING CARBOXYMETHYLATED METHIONINE RESIDUES. Compositions are expressed as amino acid residues per mole of peptide. No corrections have been applied for destruction during hydrolysis. The number of residues found by sequence analysis is shown in parenthesis.

	A1	N2	B1	B2	B2:1[a]	ε4(T)B3[a]	ε2(T)B4B[a]
Lys		1.6(1)		0.6			0.6(1)
His				0.1			
Arg	1.0(1)		1.2(1)	1.4	0.7(1)	0.7(1)	
Asp	2.0(2)			0.2			0.2(1)
Thr	1.1(1)	1.0(1)	1.8(2)	0.9		0.9(1)	
Ser	1.2(2)			1.2		1.0(1)	0.2(1)
Homoser	+	0.4	+	0.1			
Glu		1.1(1)		0.2			
Pro				0.9		0.7(1)	0.2(1)
Gly				1.1		1.1(1)	0.2(1)
Ala	2.2(2)			1.2	1.0(1)		0.2(1)
Val		1.0(1)	1.1(1)	0.4			0.4(2)
Met	+ (1)	0.2(1)	0.3(1)	0.3	0.3(1)		
Ile		0.9(1)					
Leu	1.4(1)	1.2(1)		1.0	1.0(1)		
Tyr	1.0(1)	0.8(1)					
Phe	1.3(1)						
CMMet	+	+	+	+			
Total residues	(12)	(8)	(5)		(4)	(5)	(8)

[a] calculated composition.

TABLE 19. $^{3}H/^{14}C$ RATIO FOR THE CARBOXYMETHYLATED CYSTEINS OF Fc".

Peptide	Ratio
Fc"3 CMCys 1	0.13
Fc"3 CMCys 2	0.13
Fc"2(C)N7C	0.13
Fc"2(C)A4F	5.6
Fc"2(C)A5E	82
Fc"2(C)A2C	70
Fc"2(C)N2D	3.1

TABLE 20. AMINO ACID COMPOSITION OF THE ε(ND) CHAIN. The composition is expressed as amino acid residues per M_w 62 000, the number of residues found by sequence analysis is shown in parenthesis.

Lys	19.2(21)
His	12.4(13)
Arg	30.0(31)
Asp	43.2(39)
Thr	68.1(62)
Ser	57.8(54)
Glu	46.7(47)
Pro	35.0(34)
Gly	36.2(36)
Ala	35.3(34)
Cys	15.0(15)
Val	39.7(39)
Met	7.5(7)
Ile	15.6(16)
Leu	38.0(37)
Tyr	16.6(14)
Phe	21.2(19)

DISCUSSION

All immunoglobulin (Ig) polypeptide chains can be divided into an aminoterminal portion, the variable (V) region, and the carboxyterminal portion, the constant (C) region. The V regions are associated with the antigen-binding functions and the C regions with the effector functions of an antibody molecule. According to current hypotheses (Edelman & Gall, 1969), the V and C regions are coded for by separate genes, which are joined at the DNA (or RNA) level of protein synthesis to yield a single Ig polypeptide chain. The findings that both heavy (H) and light (L) Ig polypeptide chains consist of a series of homology or domain regions, which are similar to a V region with respect to size, have led to the suggestion that V and C genes evolved from a common precursor gene that coded for a polypeptide of about 110 amino acid residues, i.e. about the length of a present day homology region. By duplication, this primordial gene gave rise to ancestral V and C genes, which by a series of genetic events (duplications, deletions, insertions etc.) generated the set of genes that code for the classes, subclasses and subgroups of contemporary V and C regions. Three classes of V regions have been recognized - V_{κ}, V_{λ} and V_{H} - which are strictly linked to the C regions of κ and λ light chains and H chains respectively. Each of these classes can be further divided into subgroups: V_{κ}(I-III), V_{λ}(I-IV) and V_{H}(I-III).

The results of the present studies on the primary structure of the heavy (ε) chain from a monoclonal IgE

protein ND provide direct support for the idea that the size of the ε chain is compatible with the presence of one V region and four C homology regions (Bennich & Johansson, 1970; Bennich & Dorrington, 1972).

As illustrated in Fig. 15, the sequence for the V region in protein ND is still incomplete with regard to about 20 amino acid residues (as indicated by brackets) in the V-C region. However, sufficient information is available to ascertain that V_{ε}(ND) is a representative of the V_HI subgroup; comparison of the sequence for the aminoterminal 95 residues in V_{ε}(ND) with "prototype" sequences for the three V_H subgroups (Gally & Edelman, 1972) gives 63, 35 and 47 percent homology between V_{ε}(ND) and V_HI, II and III respectively. The results are thus in agreement with the hypothesis that subgroup specificity of a V_H region is not restricted to any particular class of heavy chain (Putnam et al., 1972). Furthermore, by taking into consideration also the findings of another monoclonal IgE protein PS reported to belong to the V_HIII subgroup (Kochwa et al., 1971), the present results do not support the hypothesis that V_{ε} regions have characteristics differentiating them from other classes of immunoglobulins (Terry et al., 1970). The present sequence studies confirm that the heavy chain of IgE(ND) contains 15 half-cystine residues (Bennich & Johansson, 1971) of which 10 were found to participate in the formation of one intra-chain disulphide bond in each of the 5 homology regions. However, there are only three inter-chain bonds present in the ε chain ND in-

-stead of five (Bennich & Johansson, 1970); one inter-heavy-light chain bond involving Cys-120 (or Cys-121), and two inter-ε chain bonds, one at Cys-223 and the other at Cys-310, which - in analogy with inter-γ chain bonds - are presumably linked in a parallel arrangement. The structural delineation of the two remaining half-cystine residues - Cys-121 (or Cys-120) and Cys-207 - have presented problems due to their lability to mild reduction. However, by elucidation of the structure of the peptic fragment Fc" (Fig. 13) it could be demonstrated that the residues Cys-121 (or Cys-120) and Cys-207 were involved in the formation of a non-linear intra-chain disulphide bond located in the $C_{\varepsilon}1$ region. Though not previously found in a human heavy chain, the arrangement of two intra-chain bonds - one linear and the other non-linear - located within the C_H1 domain region has previously been reported for rabbit γ heavy chain (O´Donnell et al., 1970).

The attachment of a light chain to a half-cystine residue located at a homologous position to the Cys-120 in the V_{ε}-C_{ε} region has been found in heavy chains of several classes and from different species, with the exception of human γ1 and α2 heavy chains (de Préval et al., 1970; Svasti & Milstein, 1970; Perry & Milstein, 1970; Grey et al., 1968; Putnam et al., 1973). Chemical typing of IgE(PS), recently reported by Mendez (Mendez et al., 1973) confirms the labile nature of the disulphide bonds that are formed by the half-cystine residues Cys-120, Cys-121, Cys-209 and Cys-310. The finding, that the linear intra-chain bond (Cys-135 to Cys-189) in the $C_{\varepsilon}1$

region is also cleaved, is entirely compatible with the previous observation (Bennich & Johansson, 1971) that eight of the 20 disulphide bonds in native IgE(ND) are cleaved by mild reduction in the absence of unfolding agents. The fact that Mendez et al., did not identify a peptide containing the half-cystine residue Cys-223, which participates in one of the inter-ε chain bonds, does not necessarily imply that this bond (or residue) is absent in protein PS, but rather confirms the present observations (see Table 19) that the two inter-ε chain bonds are not equally susceptible to mild reduction. This relative resistance of the inter-chain bond at Cys-223 also explains the very slow production of ε chain monomers upon mild reduction (Bennich & Dorrington, 1972). In this context, it is of interest to note, that reduction has been shown to adversely effect the cytotropic activity of IgE, and the correlation observed between loss of activity and the number of disulphide bonds cleaved indicated that one of the inter-chain bonds may play an important role in conserving such activity (Stanworth et al., 1970); recently the disulphide bond at Cys-310 was advocated to fulfil this critical function (Bennich & Wahr-Lindström, 1974).

The length of the intra-chain loop in a C homology region is about 60 amino acid residues, whereas the length of the segment joining two intra-chain loops is about 45 residues. As illustrated in Fig. 16, vary these distances very little from one heavy chain class

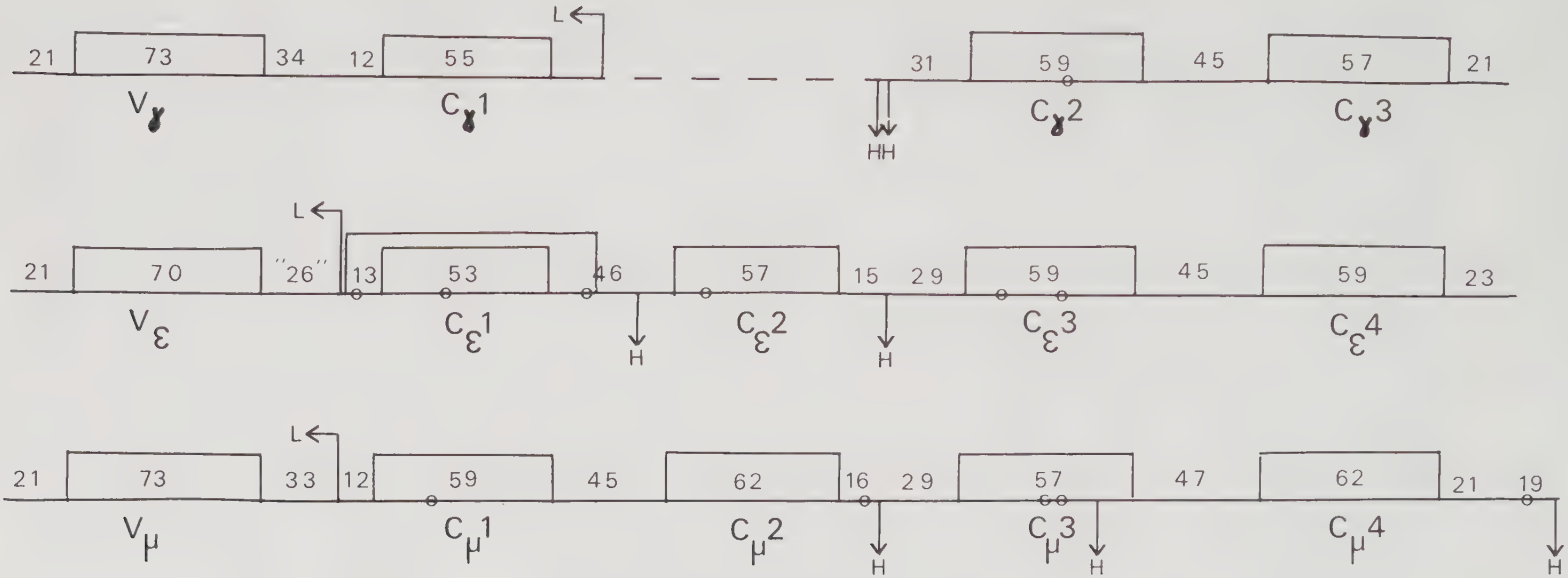


FIG. 16. Diagrammatic structures of ε(ND)-, γ(Eu)- (Edelman et al., 1969) and μ(Gal)-chains (Watanabe et al., 1973).

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γ1  C1  Cys-Leu-Val-Lys-Asp-Tyr-Phe-Pro-Glu-Pro-Val-Thr-Val-Ser-   -   -Trp-Asn-Ser-   -   -Gly-   -Ala-
    C2  Cys-Val-Val-Val-Asp-Val-Ser-His-Glu-Asp-Pro-Gln-Val-Lys-Phe-Asn-Trp-Tyr-Val-   -Asp-Gly-Val-Gln-
    C3  Cys-Leu-Val-Lys-Gly-Phe-Tyr-Pro-Ser-Asp-Ile-Ala-Val-Glu-   -   -Trp-Glu-Ser-Asn-Asp-Gly-Glu-Pro-
α   C1  Cys-Leu-Ala-Gln-Asp-Phe-Leu-Pro-Asp-Ser-Ile-Thr-Phe-Ser-   -   -Trp-Lys-Tyr-   -   -   -   -   -
    C2  Cys-Gln-Ala-Thr-Gly-Phe-Ser-Pro-Arg-Gln-Ile-Gln-Val-Ser-   -   -Trp-Leu-Arg-   -Glu-Gly-Lys-Gln-
    C3  Cys-Leu-Val-Thr-Asp-Leu-Thr-Tyr-Asp-Ser-Val-Thr-Ile-Ser-   -   -Trp-Thr-Arg-Gln-Asp-Gly-Glu-Ala-
    C4  Cys-Leu-Val-Thr-Gly-Phe-Ser-Pro-Ala-Asp-Val-Phe-Val-Gln-   -   -Trp-Gln-Met-Gln-Arg-Gly-Gln-Pro-
ε   C1  Cys-Leu-Ala-Thr-Gly-Tyr-Phe-Pro-Glu-Pro-Val-Met-Val-Thr-   -   -Trp-Asx-Thr-   -   -Gly-Ser-Leu-
    C2  Cys-Leu-Val-Ser-Gly-Tyr-Thr-Pro-Gly-Thr-Ile-Asn-Ile-Thr-   -   -Trp-Leu-   -   -Glx-Asx-Gly-Glx-
    C3  Cys-Leu-Val-Val-Asp-Leu-Ala-Pro-Ser-Lys-Gly-Thr-Val-Asn-Leu-Thr-Trp-Ser-Arg-Ala-Ser-Gly-Lys-Pro-
    C4  Cys-Leu-Ile-Gln-Asn-Phe-Met-Pro-Glu-Asp-Ile-Ser-Val-Gln-   -   -Trp-Leu-His-Asn-Glu-Val-Gln-Leu-

γ1  C1  Leu-Thr-Ser-Gly-Val-   -   -   -   -His-   -   -   -   -Thr-Phe-Pro-Ala-Val-Leu-Gln-Ser-Ser-   -Gly-
    C2  Val-   -   -   -   -   -   -   -His-   -Asn-Ala-Lys-Thr-Lys-Pro-Arg-Glu-Gln-Gln-Tyr-   -Asn-Ser-
    C3     -Glu-Asn-   -   -   -Tyr-   -   -   -   -   -Lys-Thr-Thr-Pro-Pro-Val-Leu-Asp-Ser-   -Asp-Gly-
α   C1     -Lys-Asx-Asn-Ser-Asp-Ile-Ser-Ser-   -Thr-   -   -Arg-Gly-Phe-Pro-Ser-Val-Leu-Arg-Gly-   -   -Gly-
    C2  Val-Gly-Ser-Gly-Val-   -   -   -Thr-   -Thr-Asn-   -Glu-Val-Glx-Ala-Glx-Ala-Lys-Glu-Ser-Gly-Pro-Thr-
    C3  Val-Lys-   -   -   -   -   -Thr-His-Thr-   -   -   -   -   -Asn-Ile-Ser-Glx-Ser-His-Pro-Asn-Ala-
    C4  Leu-Ser-Pro-Glu-Lys-   -Tyr-   -Val-   -Thr-Ser-Ala-   -Pro-Met-Pro-   -Glu-Pro-Gln-Ala-   -Pro-Gly-
ε   C1  Asn-Gly-Thr-   -   -   -   -   -   -Thr-   -   -   -   -Leu-Pro-Ala-Thr-Thr-Leu-Thr-Leu-Ser-Gly-
    C2  Val-Met-Asp-   -Val-Asp-Leu-   -Ser-   -Thr-   -Ala-   -Ser-Thr-   -   -Glu-Ser-Gln-Gly-Glu-Leu-Ala-
    C3  Val-Asn-   -   -   -   -   -   -   -His-Ser-Thr-   -Arg-   -Lys-   -Glu-Glu-Lys-Gln-   -Arg-Asn-Gly-
    C4  Pro-Asp-Ala-   -Arg-   -   -   -   -His-Ser-Thr-   -   -Thr-Glu-Pro-Arg-   -Lys-Thr-Lys-Gly-   -Ser-

γ1  C1  Leu-Tyr-Ser-Leu-Ser-Ser-Val-Val-Thr-Val-Pro-Ser-Ser-   -Ser-Leu-   -Gly-Thr-Gln-Thr-Tyr-Ile-   -Cys
    C2  Thr-Tyr-Arg-Val-Val-Ser-Val-Leu-Thr-Val-Leu-His-Gln-Asn-Trp-Leu-Asp-Gly-   -Lys-Glu-Tyr-Lys-   -Cys
    C3  Ser-Phe-Phe-Leu-Tyr-Ser-Lys-Leu-Thr-Val-Asp-Lys-Ser-Arg-Trp-Gln-Glu-Gly-   -Asn-Val-Phe-Ser-   -Cys
μ   C1  Lys-Tyr-Ala-Ala-Thr-Ser-Gln-Val-Leu-Leu-Pro-Ser-Lys-Asp-Val-Met-Gln-Gly-Thr-Asn-Glu-His-Val-Val-Cys
    C2  Thr-Tyr-Lys-Val-Thr-Ser-Thr-Leu-Thr-Ile-Lys-Glu-Ser-Asx-Trp-Leu-Ser-Gln-   -Ser-Met-Phe-Thr-   -Cys
    C3  Thr-Phe-Ser-Ala-Val-Gly-Glu-Ala-Ser-Ile-Cys-Glu-Asx-Asx-Trp-Asn-Ser-Gly-   -Glu-Arg-Phe-Thr-   -Cys
    C4  Arg-Tyr-Phe-Ala-His-Ser-Ile-Leu-Thr-Val-Ser-Glu-Glu-Glu-Trp-Asn-Thr-Gly-   -Glu-Thr-Tyr-Thr-   -Cys
μ   C1  His-Tyr-Ala-Thr-Ile-Ser-Leu-Leu-Thr-Val-   -Ser-Gly-Ala-Trp-Ala-Lys-   -   -Gln-Met-Phe-Thr-   -Cys
    C2  Ser-Thr-Glu-Ser-Gln-Leu-Thr-Leu-Ser-   -Gln-Lys-   -His-Trp-Leu-Ser-Asp-   -Arg-Thr-Tyr-Thr-   -Cys
    C3  Thr-Leu-Thr-Val-Thr-Ser-Thr-Leu-Pro-Val-Gly-Thr-Arg-Asp-Trp-Ile-Glu-Gly-   -Glu-Thr-Tyr-Gln-   -Cys
    C4  Gly-Phe-Phe-Val-Phe-Ser-Arg-Leu-Glu-Val-Thr-Arg-Ala-Glu-Trp-Gln-Glu-Lys-   -Asp-Glu-Phe-Ile-   -Cys
```

FIG.17 . Comparison of the sequences of the domain regions of the ε(ND)-, γ(Eu)- (Edelman et al., 1969) and μ(Gal)-chains (Watanabe et al., 1973).

to another, with the exception of the hinge region in the γ heavy chain. The term "hinge" region originally used to indicate a stretch of the γ chain whose presumably "loose" structure contributed to an apparent flexibility of the compact globular Fab regions relative to the Fc region, has also been defined as the sequence joining the Fd and Fc segments of an heavy chain. The fact that the γ hinge region contains all the inter-γ chain disulphide bonds has provided an argument for the use of the alternative term "bridge" region (Svasti & Milstein, 1972). For a study of the homology between ε, γ and μ chain sequences, the intra-chain loops from their C regions were compared as shown in Fig. 17 . The results, given as percent homology, are summarized in Table 21. The sequences (in Fig. 17) were also compared with respect to the number of base changes required to transcribe one sequence into the other, and these results are shown in Table 22.

If it is assumed that an ancestral prototype for the C region consisted of 4 homology regions, the presence of only 3 homology regions in a heavy chain, e.g. the γ chain, indicates that one of the domain regions in the ancestral C region has been deleted at some time during the evolution of the heavy chain classes. On the basis of the structural homology of the C_H1 region in the three heavy chains and the homology present between the $C_\gamma2$ - $C_\gamma3$ and the C_H3 - C_H4 of the ε and μ chains (Fig. 17) it could be argued that any features reminiscent of such a deleted domain region are likely to be found within the hinge region of the

TABLE 21. Comparison of the degree of homology (in percent) between the domain regions in the ε(ND)-, γ(Eu)- and μ(Gal)-chains as aligned in Fig.17 .

	$C_\gamma 1$	$C_\gamma 2$	$C_\gamma 3$	$C_\mu 1$	$C_\mu 2$	$C_\mu 3$	$C_\mu 4$
$C_\varepsilon 1$	32	21	25	18	27	14	29
$C_\varepsilon 2$	17	15	19	13	23	20	23
$C_\varepsilon 3$	23	33	25	13	23	23	28
$C_\varepsilon 4$	19	25	32	14	22	10	26
			$C_\gamma 1$	33	23	20	29
			$C_\gamma 2$	11	23	18	29
			$C_\gamma 3$	19	22	17	33

TABLE 22. Differences between the analogous domain regions in the ε(ND)-, γ(Eu)- and μ(Gal)-chains as aligned in Fig. 17. The differences are calculated as minimum number of base changes required to convert one sequence into the other. Gaps are computed as two base changes.

	C1	C2	C3	C4
ε vs γ	61		59	69
ε vs μ	75-81	67-72	63-65	72-73
μ vs γ	62-65		68-70	58-59

TABLE 23. The carbohydrate composition of native IgE(ND) (Clamp & Johnson, 1972) as compared with the composition calculated from the data on the constituent glycopeptides.

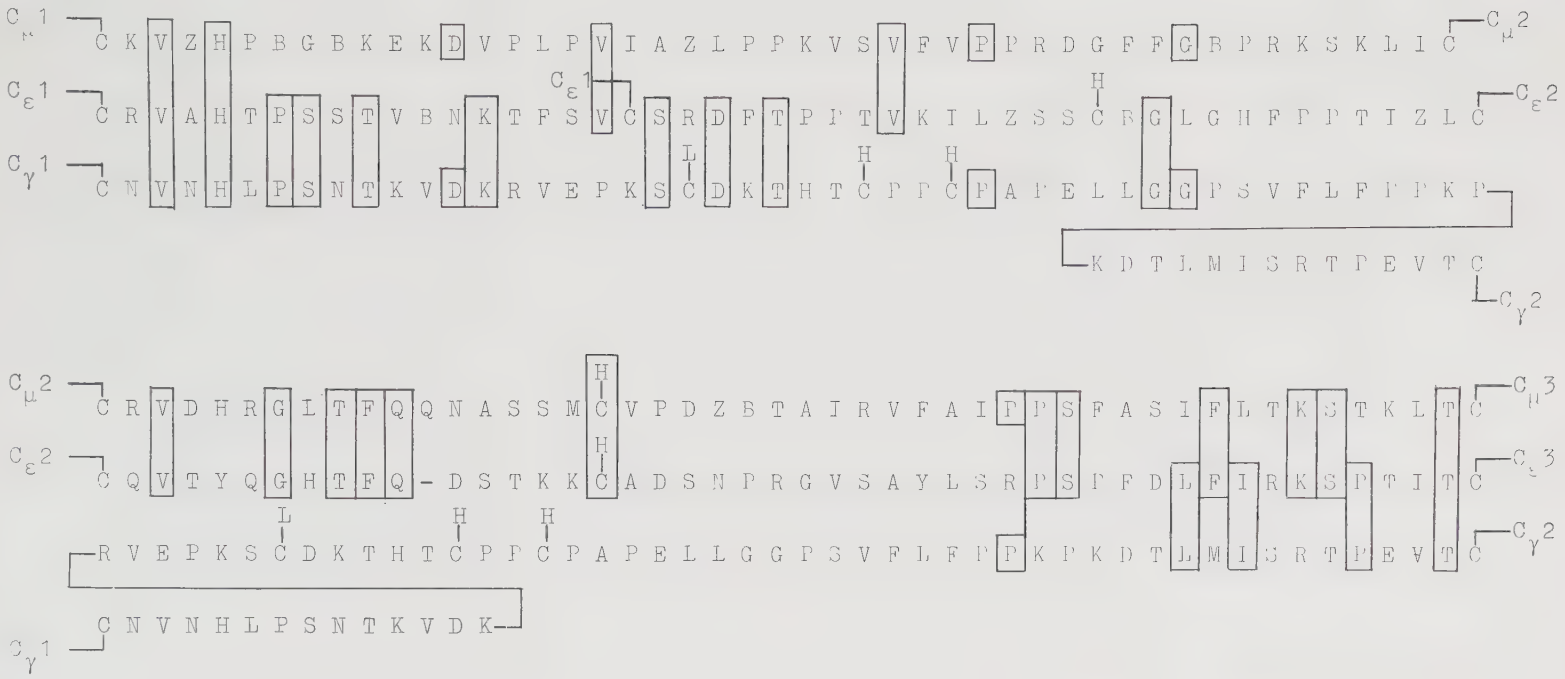
	Fucose	Mannose	Galactose	N-acetyl-glucosamine	Sialic acid
CHO:A-F	7.0	45.0	25.6	41.8	12.2
IgE(ND)	7	44	22	45	9
	4	36	17	36	18

present day γ heavy chain. Accordingly, a gap was introduced in the hinge region when the ε, γ and μ chains were aligned as illustrated in Fig. 16. In concert with this assumption might be the findings that subclass differences in IgG are restricted to the hinge region (Frangione et al., 1969a, 1969b), which further suggests that the gene(s) for this segment must have been exposed to a unique evolutional pressure.

In contrast to the μ chain, the ε chain has two inter-domain regions containing an inter-heavy chain disulphide bond, one located between $C_\varepsilon 1$ and $C_\varepsilon 2$ and the other between $C_\varepsilon 2$ and $C_\varepsilon 3$. The position of the latter bond is homologous to the position of the inter-μ chain bond located in the segment joining the $C_\mu 2$ and $C_\mu 3$ regions. The homology of the inter-$C_H 2$-$C_H 3$ region from ε and μ chains is 26 percent, in contrast to the homology of their inter-$C_H 1$-$C_H 2$ regions which is only about 9 percent. Although these inter-domain regions show no or little homology with the γ hinge region, an examination of the location of half-cystine residues and the distribution of Pro- and in particular Pro-Pro-containing peptides, indicates as shown in Fig. 18 that the aminoterminal portion of the γ hinge region would be more related to an inter-$C_H 1$-$C_H 2$ region, whereas its carboxyterminal half would appear more related to an inter-$C_H 2$-$C_H 3$ region.

The great variation of inter-heavy chain half-cystine residues seen in heavy chains of different classes contrasts sharply to the otherwise conservative distribution of intra-chain residues within Ig polypeptide chains.

FIG. 18. Comparison of the sequences of the inter-C_H1-C_H2, inter-C_H2-C_H3 regions of the ε(ND)-, μ(Gal)-chains and the γ(Eu)-hinge region.



Furthermore the sequences around the intra-chain cysteines are in general very similar as can be seen in Fig. 19, and particularly striking is the great homology for analogous peptide segments. An exception to this "rule of symmetry" is provided by the observation that an identical hexapeptide - Met-Phe-Thr-Cys-Arg-Val - contains the carboxyterminal intra-chain cysteine residue in both the $C_{\varepsilon}1$ and $C_{\mu}2$ regions.

IgE is rich in carbohydrate - about 12 percent (Bennich & Johansson, 1971; Kochwa et al., 1971) - and six oligosaccharide side chains have been identified in the ε heavy chain ND: One is located in the V-C region attached to Asn-127, one in the $C_{\varepsilon}1$ region (Asn-155), one in the first bridge region (Asn-201), one in the $C_{\varepsilon}2$ region (Asn-248) finally two in the $C_{\varepsilon}3$ region (Asn-353 and Asn-376). In Table 23 the total composition of sugar residues as determined by analysis of the native protein ND (Bennich & Johansson, 1971) is compared with the composition calculated from the present analyses of the constituent glycopeptides. Although there is a discrepancy in the compositions reported, the variation for the relative values is relatively small, with the exception of the value for sialic acid, which was very high in the composition obtained by Clamp and Bennich (Clamp & Johnson, 1972). The most likely explanation of this difference is that sialic acid residues may have been lost during the preparation of fragments and peptides for sequence analysis. The carbohydrate composition and the amino acid sequence of the glycopeptide regions from ε and μ chains are compared in Table 24. The pre-

FIG. 19. Comparison of the sequences around the intra-chain disulphide bonds of the ε(ND)-, γ(Eu) (Edelman et al., 1969) and γ(Gal)-chains (Watanabe et al., 1973).

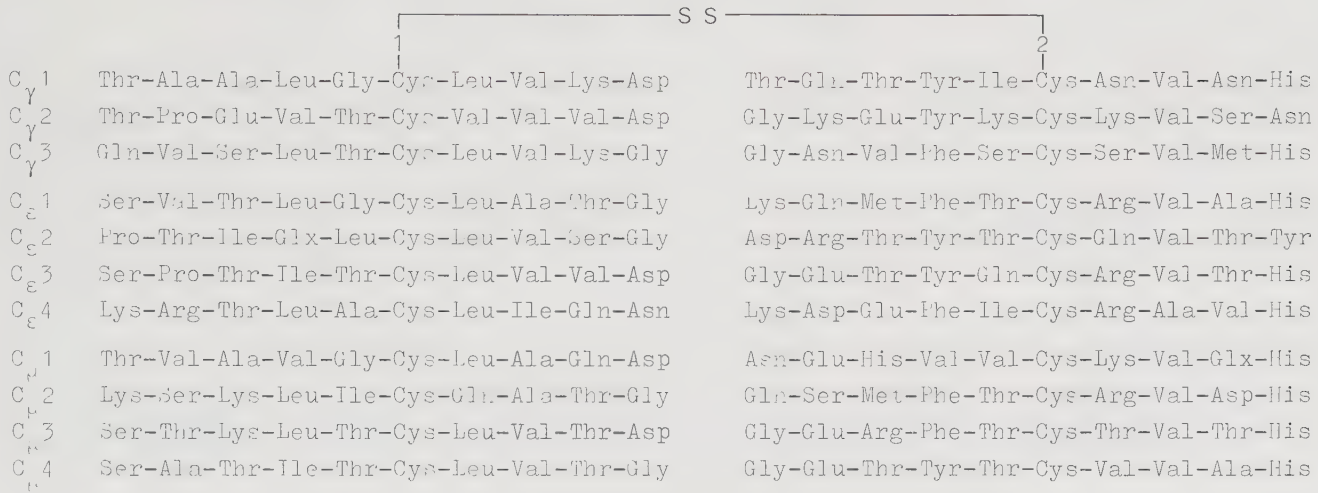


TABLE 24. Glycopeptides of human μ- and ε-chains. Monosaccharides are expressed as residues per mole of peptide. The sequence data and the carbohydrate composition of the μ-chain is taken from Shimizu *et al.* (1971) and Putnam *et al.* (1973).

	Fucose	Mannose	Galactose	-acetyl-glucosamine	Sialic acid	Glycopeptides
ε(ND)						
CHO:A	1.3	3.3	2.5	3.9	0.5	-Pro-Ser-Asn-Ala-Thr-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.7	-Ser-Leu-Asn-Gly-Thr-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-Val-Asx-Asn-Lys-Thr-
CHO:D	trace	3.0	2.4	3.5	2.1	-Thr-Ile-Asn-Ile-Thr-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-Thr-Val-Asn-Leu-Thr-
CHO:F	0	5.7	0.3	2.0	0	-Gln-Arg-Asn-Gly-Thr-
μ						
C-1	1	2-3	2	3		-Tyr-Lys-Asn-Asn-Ser-
C-2	1	2-3	2	3-5		-Gln-Gln-Asn-Ala-Ser-
C-3	1	3	2	4-5		-His-Thr-Asn-Ile-Ser-
C-4	0	5	0	2		-His-Pro-Asn-Ala-Thr-
C-5	0	8-11	0	2-3		-Leu-Tyr-Asn-Val-Ser-

-sence of carbohydrate units of both complex and simple structure has previously been observed in the μ chain (Shimizu et al., 1971). In contrast to ε and μ chains, the γ chain contains only one oligosaccharide side chain, which represents the complex type of structure (Clamp [illegible], [illegible]). A comparison of the location of carbohydrate side chains in the ε and μ chains, reveals no obvious homology except for the positions of the units in C_H1 and the simple side chains in the C_H3-intra-chain loop; the latter are furthermore homologous in position to the complex oligosaccharide side chain attached to the γ heavy chain.

Baenziger et al. (1974) have recently reported the structure of the oligosaccharide in [illegible]. Their data differs from ours with respect to the number of sialic acid-containing side chains - only 4 complex units were found - but confirms the presence of one simple type oligosaccharide unit in the ε chain. The structure of the latter is interesting since it includes an α-linked N-acetyl-glucosamine, but at present it is difficult to evaluate what, if any, significance this - for a serum glycoprotein - unique structure may have for the function of IgE.

SUMMARY

The amino acid sequence of the ε heavy chain from a monoclonal IgE protein ND has been determined. The ε chain ND which consists of about 530 residues (the sequence for about 20 residues in the V-C region is still incomplete) contains 5 homology regions, one in the variable (V) region and four in the constant (C) region. The sequence of the V_{ε}(ND) region is homologous (63 percent) to proteins of the V_HI subgroup. The $C_{\varepsilon}1$ region contains two intra-chain disulphide bonds, one arranged in a linear and the other in a non-linear fashion, which is homologous to the arrangement of disulphide bonds in the rabbit $C_{\gamma}1$ region. There are 3 inter-chain bonds in ε chain ND: the one representing the bond between a light (λ) and the ε chains is located in the V-C region, whereas the two representing inter ε chain bonds are located in inter domain regions $C_{\varepsilon}1$-$C_{\varepsilon}2$ and $C_{\varepsilon}2$-$C_{\varepsilon}3$ respectively. Six oligosaccharide side chains have been located. The composition of sugar residues indicated that five of the carbohydrate units have a complex and one has a simple type of structure.

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